CHEMOPATHOLOGICAL STUDIES WITH COMPOUNDS OF ARSENIC.

I. Types of the Arsenic Kidney.

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Plates 57 to 60.

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During the past year, while investigating the pathological action of a large number of organic preparations of arsenic,[1] it was noted that the gross appearance of many of the kidneys resulting from the administration of these compounds did not conform to the type usually described as the arsenic kidney. The wide variations in the type of kidney observed, and at the same time the constancy of the type produced by a given compound in a given animal species, were so marked as to suggest that the classical picture was not a constant one for all compounds of arsenic. To obtain a more comprehensive idea of these variations and some conception of their cause, a series of experiments was carried out with a number of well known arsenicals, and the gross pathological results obtained serve as the basis for this report. The microscopical studies are reported in a separate paper.

EXPERIMENTAL.

Dogs were used for these experiments, because the dog kidney is large enough to show the gross pathological picture quite well. This series of experiments was supplemented, however, by similar experiments carried out with guinea pigs, intended principally as a

[1] For several years, The Rockefeller Institute for Medical Research has been interested in the chemotherapy of infections. The work now in progress is being carried out jointly by a chemical division and a biological division. The chemical work is being done by Dr. W. A. Jacobs and Dr. Michael Heidelberger. The series of studies contemplated under the title "Chemopathological Studies with Compounds of Arsenic" are an outgrowth of the work on chemotherapy.

control upon the histological changes produced, since the dogs obtainable for this work so frequently showed spontaneous kidney lesions.[2] It may be stated here that the gross appearances presented by the guinea pig kidneys were essentially the same as those of the dogs.

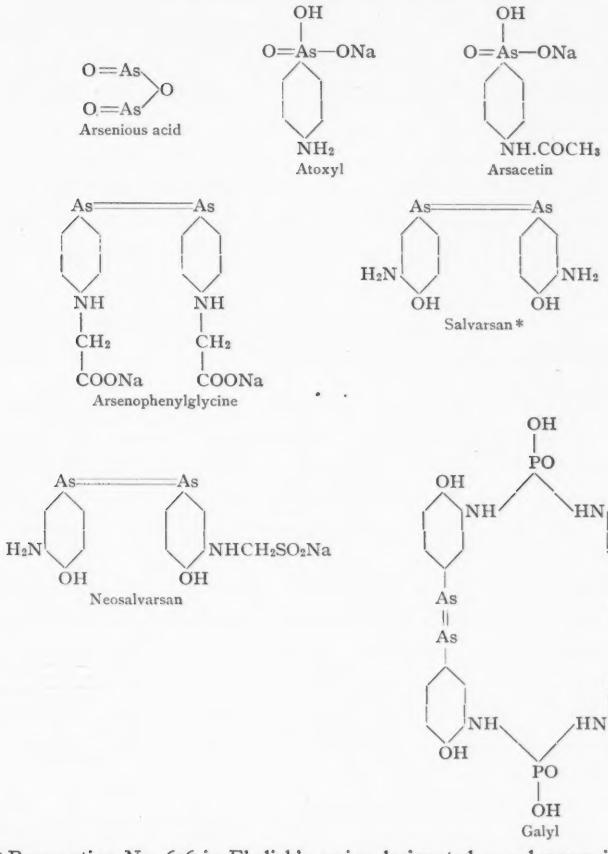
TABLE I.

Dog.	Drug.	Dose per kilo.	Strength of solution.	Route of administration.	Remarks.					
					1 day.	2 days.	3 days.	4 days.	5 days.	6 days.
		mg.								
A	Arsenious acid	7	1:100	Subcutaneous	Dead					
B	Arsenious acid	5	1:333	Intraperitoneal	"					
C	Atoxyl	40	1:50	Subcutaneous	Slightly ill	Well	Well	Weak	Better	Dead
D	"	20	1:10	Subcutaneous	Well	"	40 mg.	Ill	Killed	
E	"	50	1:50	Intraperitoneal	Ill; killed					
F	"	20	1:75	Intraperitoneal	" "					
G	Arsacetin	300	1:10	Intraperitoneal	Ill	Better	Better	Well 600 mg.	Dead	
H	"	400	1:25	Intravenous	Ill; killed					
I	"	600	1:10	Intravenous	" "					
J	"	400	1:40	Intravenous	Ill	Dead				
K	Arsenophenylglycine	225	1:10	Intraperitoneal	"	Better	3 days. Well 225 mg.		19 days. Well 250 mg.	20 days. Well; killed.
L	Arsenophenylglycine	500	1:10	Intravenous	Dead					
M	Arsenophenylglycine	300	1:20	Intravenous	"					
N	Arsenophenylglycine	200	1:20	Intravenous	Well; killed					
O	Arsenophenylglycine	500	1:15	Intravenous	Dead					
P	Salvarsan	100	1:400	Intraperitoneal	Ill	Ill; killed				
Q	"	100	1:333	Intravenous	Dead					
R	Neosalvarsan	150	1:30	Intravenous	"					
S	Galyl	100	1:100	Intravenous	"					

[2] Dayton, H., *Jour. Med. Research*, 1914, xxxi, 177.

TABLE II.

Structural Formulæ of Arsenicals.



* Preparation No. 606 in Ehrlich's series designated as salvarsan is the dihydrochloride of this compound. The formula here given, however, is that most frequently encountered in the literature and is the substance derived from the dihydrochloride by the addition of alkali.

Solutions of arsenious acid, atoxyl, arsacetin, arsenophenylglycine, salvarsan, neosalvarsan, and galyl were injected without an anesthetic into young adult dogs. The injections were made intravenously, intraperitoneally, or subcutaneously; no difference was

noted in the results obtained by using these different routes. Doses of various size were used, as shown in Tables I and II, but in the majority of instances amounts approximating the lethal dose were injected, in order to accentuate the lesion produced by the particular compound. In a few instances much smaller amounts were used, in order to determine the point of greatest susceptibility and the characteristics of the essential lesions. The animals were usually killed at the end of twenty-four hours, if they were not already dead, so that we might obtain lesions comparable in time (length of survival) as well as dose. In each instance the dog was lightly anesthetized with chloroform and bled to death from the carotid artery.

RESULTS.

Arsenious Acid.

Injection of arsenious acid into dogs produces a uniformly red kidney (Fig. 1). The organ is enlarged, is purplish red in color, and on section drips blood. The cut surface is reddened from the outermost edge to the pelvis, through the cortex, boundary zone, and medulla. All the zones or territories of the kidney are practically uniformly involved. The kidneys of dogs that survive large doses of arsenious acid for a sufficient length of time show opaque, yellowish white striations in the inner portion of the cortex resulting from tubular necrosis which, however, is not an essential feature of the change. The outspoken picture is that of a diffusely reddened kidney indicative of uniform vascular injury and is one of the two principal types produced by arsenical compounds.

Salvarsan, Neosalvarsan, and Galyl.

The type of kidney produced by injections of these drugs falls into the general class of red kidneys, although certain differences relate them to the other main type, the pale kidney. These differences consist in a relatively greater degree of tubular necrosis and in the character of the vascular injury. This group of kidneys, as shown by the salvarsan and neosalvarsan kidneys (Figs. 3 and 8, respectively), shows a considerable amount of necrosis of the cortex which is most conspicuous in the inner half. On the other hand,

although the kidney is reddened throughout, the congestion and hemorrhage are most prominent in the outer half of the cortex and the inner portion of the boundary zone.

Arsacetin.

Injection of arsacetin into dogs produces an exceedingly pale kidney (Fig. 2), that differs strikingly from the red type just described. The organ is enlarged, soft, and very friable. It is grayish or yellow in color; in some instances it may be pink, with slight congestion of the surface vessels. It cuts very easily and on section the cut edge is everted. The cortex bulges, is widened, and the striations are yellowish, opaque, and raised as a result of wide-spread tubular necrosis; the glomeruli may or may not be prominent. The boundary zone practically always shows some congestion and the same may be said of the medulla, but to a much less degree. The dominant feature of this type of kidney, therefore, is its paleness due to tubular necrosis, as contrasted with the redness of the kidney of arsenious acid resulting from vascular injury. Kidneys of dogs that survive large doses of arsacetin for a sufficient length of time show a greater degree of congestion or even some hemorrhage in the boundary zone. The hemorrhage may extend somewhat into the medulla and to a lesser degree into the cortex.

Arsenophenylglycine and Atoxyl.

Both of these drugs produce pale types of kidneys, but the amount and extent of vascular injury are somewhat greater than in the arsacetin kidney. The cortical necrosis of the arsenophenylglycine kidney (Fig. 4) is extreme and is quite comparable to that produced by arsacetin. On the other hand, the congestion and hemorrhage of the medulla are relatively prominent and especially so in the inner portion of the boundary zone. We may, therefore, consider the kidneys produced by these drugs as related to both the pale and the red types of kidneys.

DISCUSSION.

In studying the action of this group of arsenicals upon the kidney, the object in view has necessitated the subordination of detail to the

broader conception of types of renal injury. Further, we can not now attempt a complete description and grouping of all the types of arsenic kidneys which we have observed. Our object, at present, is to lay emphasis upon the difference in the character of the renal injury produced by different compounds of arsenic as expressed in the gross appearance of the organ. For this purpose we have chosen two extreme types.

The classical red kidney resulting from injections of arsenious acid into dogs contrasts sharply with the pale kidney produced by arsacetin. The red kidney is one in which uniform vascular injury predominates; all zones of the kidney seem to be affected equally, cortex as well as medulla. The pale kidney, on the other hand, presents striking evidence of the dominance of tubular necrosis, and the reddening of the boundary zone is a relatively inconspicuous feature. With the other arsenicals this difference in the gross appearance of the kidneys is not so marked, and we may perhaps look upon the kidneys produced by salvarsan, neosalvarsan, and galyl, on the one hand, and by atoxyl and arsenophenylglycine on the other, as constituting transitions or subgroups between the two principal types. The salvarsan group of kidneys is preeminently red, but there is also a relatively great degree of tubular necrosis; the arsenophenylglycine group of kidneys is pale, yet it shows relatively marked vascular injury, especially in the boundary zone and contiguous territory.

The type of kidney produced by a given compound can not be regarded as fixed in all its details, but the size of the dose and the rate of action as measured by the length of survival of the animal are factors that exercise a distinct influence upon the final result, as may be illustrated by atoxyl. The lethal effects of atoxyl are developed slowly in the dog and it would probably require many times the minimal lethal dose to kill within twenty-four hours. The tolerable dose of this drug for dogs as given by Mesnil and Brimont[3] is 10 mg. per kilo of body weight. Dog F, given double this dose (20 mg. per kilo), showed slight symptoms of intoxication at the end of twenty-four hours and when killed the kidneys were quite pale and slightly enlarged. The cortex showed opaque striations,

[3] Mesnil, F., and Brimont, E., *Ann. de l'Inst. Pasteur,* 1908, xxii, 856.

but the striking feature was a pencil line of hemorrhage in the inner boundary zone (Fig. 6). This animal illustrates well the character and location of the essential changes produced by atoxyl. Dog E, given 50 mg. of atoxyl per kilo, was more toxic at the end of twenty-four hours and was killed. The kidneys (Fig. 7) were markedly enlarged and the opaque yellow gray surface was thickly stippled with red. On section the cortex showed alternate striations of red and opaque yellowish gray; the glomeruli were prominent. The line of red in the medulla of Dog F had given place to an intense reddening of the entire medulla, which was practically a mass of clotted blood. The massive dose of atoxyl used in this dog exaggerates the effects produced in Dog F and at the same time serves to indicate the character of changes referable to dosage. Dog C received 40 mg. of atoxyl per kilo and although this animal passed large amounts of blood in the urine, other evidence of intoxication was relatively slight. It finally died on the sixth day. The kidneys of this dog were practically identical with those of Dog D (Fig. 5) which received a dose of 20 mg. per kilo, three days later a second dose of 40 mg. per kilo, and was then killed after two days. With these two dogs the influence of time as well as dose is introduced. This group of atoxyl kidneys illustrates well the elasticity of a type but at the same time emphasizes the sharp differentiation of even a transitional group from the classical arsenious acid kidney.

As regards the factors that endow compounds of arsenic with the power of producing constant and characteristic types of renal injury, one can hardly escape the conclusion that such activities are intimately related to the chemical constitution of the compounds. However, both organic and inorganic compounds of arsenic in which the arsenic is trivalent or pentavalent are known to produce red kidneys of the arsenious acid type. On the other hand, while both trivalent and pentavalent compounds of arsenic produce pale kidneys, we know of no inorganic arsenical that acts in this way. Further, substances as closely related as atoxyl and arsacetin, or as salvarsan and arsenophenylglycine, may act quite differently. Again, compounds as different as arsenious acid and salvarsan, or as arsacetin and arsenophenylglycine, may simulate each other quite closely in their effects.

For the present, therefore, we can go no further than the conclusion that the character of the renal injury produced by compounds of arsenic is determined either directly or indirectly by the chemical constitution of the compound.

CONCLUSIONS.

1. All arsenical compounds do not produce the same type of renal injury.

2. In general, there are two broad groups of kidneys produced, the red and the pale, with a variety of subdivisions of each group depending upon modifications in the chemical constitution of the compound, dosage, and length of survival of the animal.

EXPLANATION OF PLATES.

PLATE 57.

FIG. 1. Kidney of arsenious acid (natural size). Dog A. Red type of kidney. Uniform congestion and hemorrhage throughout cortex and medulla. Tubular necrosis in inner half of cortex.

FIG. 2. Kidney of arsacetin (natural size). Dog I. Pale type of kidney. Necrosis of cortex. Congestion of boundary zone.

PLATE 58.

FIG. 3. Kidney of salvarsan (natural size). Dog Q. Reddening of kidney throughout, especially of outer cortex and inner portion of boundary zone. Tubular necrosis of inner half of cortex.

FIG. 4. Kidney of arsenophenylglycine (natural size). Dog L. Tubular necrosis of entire cortex. Diffuse congestion of medulla with a band of hemorrhage.

PLATE 59.

FIG. 5. Kidney of atoxyl (natural size). Dog D. Necrosis of cortex. Diffuse congestion of medulla with hemorrhage of boundary zone and inner half of cortex.

PLATE 60.

FIG. 6. Kidney of atoxyl (reduced). Dog F. Pencil line of congestion and hemorrhage along inner boundary zone.

FIG. 7. Kidney of atoxyl (reduced). Dog E. Necrosis of cortex. Hemorrhage of medulla.

FIG. 8. Kidney of neosalvarsan (reduced). Dog R. Necrosis of cortex. Congestion and hemorrhage of boundary zone.

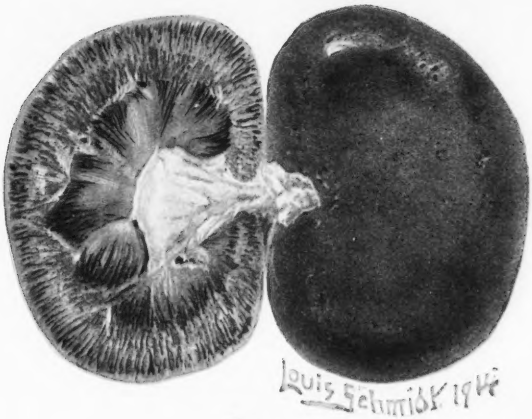


FIG. 1.

FIG. 2.

(Pearce and Brown: Types of Arsenic Kidney.)

FIG. 3.

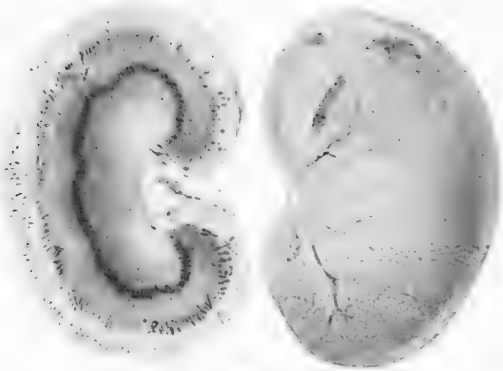


FIG. 4.

(Pearce and Brown: Types of Arsenic Kidney.)

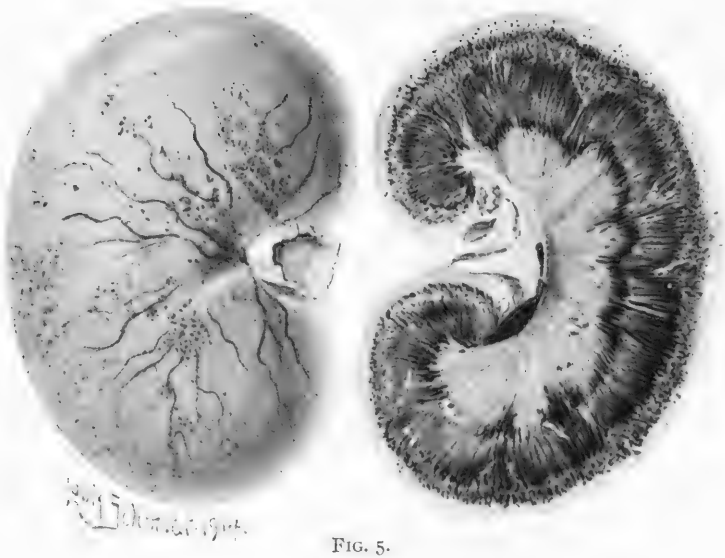


FIG. 5.

(Pearce and Brown: Types of Arsenic Kidney.)

FIG. 6.

FIG. 7.

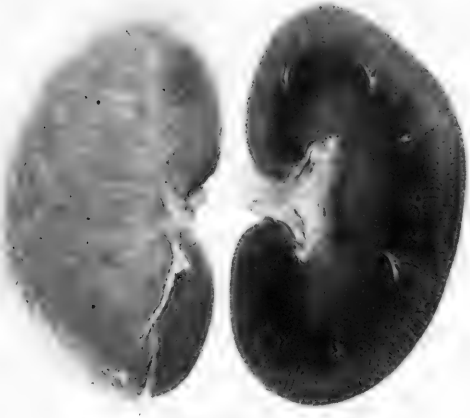


FIG. 8.

(Pearce and Brown: Types of Arsenic Kidney.)

CHEMOPATHOLOGICAL STUDIES WITH COMPOUNDS OF ARSENIC.

II. Histological Changes in Arsenic Kidneys.

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Plates 61 to 67.

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The idea that arsenicals as a class produce a vascular type of injury in the kidney has gained general acceptance, and workers in experimental nephritis have substantiated this idea for such compounds as have been employed to produce so called arsenical nephritis. However, as we have shown in the preceding paper,[1] all compounds of arsenic do not produce the same type of injury, and from the gross appearance of the kidneys of dogs poisoned with various well known arsenicals, we are able to recognize two widely separated types of kidney, the red and the pale, with many transitional varieties or subgroups, each being more or less characteristic for a given compound. In like manner, we have found that the histological changes in these given types are equally different and characteristic.

EXPERIMENTAL.

Tissues from the kidneys described and illustrated in the preceding paper[2] were fixed in Zenker's fluid, sectioned in paraffin, and stained with hematoxylin and eosin. The histological changes, therefore, may be related directly to the descriptions and illustrations in this paper.

Red Kidneys.

Arsenious Acid.—The most characteristic acute lesions produced by arsenious acid consist in a uniform dilatation and congestion of

[1] Pearce, L., and Brown, W. H., *Jour. Exper. Med.*, 1915, xxii, 517.

[2] Pearce and Brown, *loc. cit.*

the blood vessels of the kidney with an escape of blood into the interstitial tissues throughout the cortex and medulla (Fig. 1). The glomeruli are swollen, and the tuft completely fills the capsular space. Occasionally there is desquamation of swollen, disintegrating cells of the capsular epithelium and a slight albuminous precipitate in the capsular space. The tuft vessels are widely dilated and filled with blood; some contain hyaline plugs. The changes in the tubular epithelium are less marked, the cells are greatly swollen and vacuolated, often occluding the lumen of the tubule and are frequently stripped up from the basement membrane. Parenchymatous and fatty degeneration are marked, but necrosis of tubular epithelium is relatively slight. Some of the tubules contain an albuminous precipitate; others, especially in the boundary zone, contain blood.

Salvarsan.—While vascular injuries dominate the histological changes produced by poisoning with salvarsan, the lesions differ in essential respects from those produced by arsenious acid. Throughout the cortex and medulla the vessels are dilated and congested, but patches of hemorrhage are more numerous in the outer cortex and are especially prominent in the boundary zone. The glomeruli are all large, with the tuft practically filling the capsular space; the tuft capillaries are widely dilated and filled with blood; and there is a slight accumulation of polymorphonuclear leucocytes in the capillaries. The cells of the outer portion of many of the tufts are markedly swollen, granular, and pale-staining, and many of these swollen cells appear to be desquamating into the capsular space (Fig. 4). Hyaline thrombi are numerous (Fig. 3). Occasionally there is a slight amount of albuminous precipitate in the capsular space. The changes in the tubules are relatively more pronounced than with arsenious acid. The epithelium of the tubules shows an extreme degree of parenchymatous and fatty degeneration with definite necrosis. The cells are very granular and ragged with frayed edges, and many are actually disintegrating (Figs. 3 and 4). In many areas there is a protrusion of swollen tubular epithelium into the glomerular space. Practically all the tubules contain albuminous precipitate and some of the cortical and many of the medullary tubules contain blood (Fig. 3). Of especial interest is a fairly extensive and regular edema of the labyrinth, particularly of the

lower two-thirds of the cortex extending but little into the medulla (Fig. 4).

Neosalvarsan.—The histological changes produced in the kidney by neosalvarsan are still further removed from those produced by arsenious acid, both in type and degree, although they conform in general to the changes observed in other red kidneys. Here again vascular dilatation and congestion are present, and hemorrhage, as with salvarsan, is most marked in the outer cortex and boundary zone.

The glomeruli are large, but the tuft itself is small and compressed, occupying approximately only one-third to one-half of the capsular space. The remaining space is filled with an extensive albuminous precipitate (Fig. 5). The tuft vessels are irregular, many being enormously dilated and filled with blood and numerous hyaline plugs; in consequence, other vessels are completely collapsed. There is an increase in the number of polymorphonuclear leucocytes in the capillaries of the tuft. The injury to the tubular epithelium is distinctly more prominent than with either arsenious acid or salvarsan. The epithelium of the convoluted tubules, particularly of the outer half of the cortex, is almost completely necrotic, the epithelium of many of the tubules being converted into homogeneous, pink-staining masses (Fig. 5). There is less tubular necrosis in the inner cortex, but there is marked parenchymatous and fatty degeneration with an albuminous precipitate in the lumen of the tubules. A considerable number of the ascending limbs of the loops of Henle shows an extreme degree of degeneration occasionally going on to necrosis. Many of the tubules in the boundary zone and medulla contain blood. In the lower portion of the cortex and in the boundary zone there are a few irregular patches of interstitial edema.

Galyl.—In general, sections from kidneys after injection of galyl resemble those of salvarsan and neosalvarsan, but there are certain relative differences. The tuft capillaries are uniformly dilated and contain blood and irregularly distributed hyaline thrombi. There is a considerable amount of swollen cells desquamated from the capsule into the capsular space (Fig. 2). The epithelium of the convoluted tubules, especially in the outer half of the cortex, shows disintegration, desquamation, and necrosis, but there is very little necrosis *en masse*. The epithelium of the loops of Henle shows de-

generation, although comparatively little necrosis. The cortical tubules contain much albuminous precipitate, and the majority of the tubules of the medulla contain blood.

Pale Kidneys.

Arsacetin.—In the group of pale kidneys, of which the arsacetin kidney is taken as the type, the relative degree of vascular and tubular injury is the reverse of that observed in the red kidney. Following the injection of arsacetin, the vessels and capillaries of the kidney are not usually dilated or congested except to a slight degree in the boundary zone, and there is no hemorrhage. The glomeruli are practically normal in appearance; the tuft fills most of the capsular space but is not swollen; the capillaries of the tuft are moderately dilated and contain some blood. There is only a slight amount of albuminous exudate. On the other hand, practically all the epithelium of the convoluted tubules is necrotic, the tubules appearing as large, swollen, granular, pink-staining masses (Fig. 6). The cells which are not actually necrotic are extremely degenerated with pyknotic and fragmenting nuclei. The tubules are choked with masses of disintegrating cells or contain an albuminous precipitate. The epithelium of the loops of Henle is markedly degenerated, the cells are swollen, ragged, and vacuolated. In some instances, when a very large dose of arsacetin is given (Dog I),[3] the wide-spread necrosis of tubular epithelium includes that of the ascending limb of the loop of Henle. The collecting tubules show a moderate amount of parenchymatous and fatty degeneration.

Arsenophenylglycine.—The injury produced in the kidney by arsenophenylglycine combines both vascular and tubular changes. The vessels and capillaries throughout the kidney are moderately dilated and congested. There is slight escape of blood, if any, from the vessels in the cortex, but in the boundary zone there is a considerable amount of hemorrhage which extends in streaks into the medulla. The glomeruli are uniformly large, the tuft filling approximately one-third to one-half the capsular space. The capillaries of the tuft are irregular in appearance; some are moderately

[3] The designations of the dogs correspond to those given in the preceding paper (Pearce and Brown, *loc. cit.*).

dilated and filled with blood, but many are completely collapsed. The epithelium of the capsule and of the adjoining portion of the connecting tubule is enormously swollen, homogeneous, and pale pink. Many cells of the capsule and connecting tubule have desquamated into the capsular space (Fig. 7), and in certain instances the mass of degenerated and disintegrating cells in the space apparently comes almost entirely from the tubule. Practically all the convoluted tubules are necrotic and appear as solid, homogeneous, pink-staining cylinders, or the tubule is filled with disintegrating hydropic cells. The epithelium of the loops of Henle in the outer cortex is similarly necrotic but in the inner portion there is less actual necrosis. Here the cells are markedly swollen and granular with many large vacuoles and pyknotic nuclei. The tubules contain an albuminous precipitate. The epithelium of the collecting tubules is also degenerated. The lumen of almost all the tubules is completely occluded by necrotic cell masses or by enormously swollen, degenerated, and desquamated cells. In the medulla hyaline casts are quite abundant and many tubules contain blood. There are a few irregular patches of interstitial edema in the inner cortex.

Atoxyl.—Following a small dose of atoxyl (Dog F), the only distinctive pathological changes consist in parenchymatous and fatty degeneration of tubular epithelium and slight congestion and hemorrhage in the boundary zone. The epithelium of the convoluted tubules stains palely, the cells are ragged and granular with irregular vacuolization, as shown in Fig. 8. There is some fragmentation of nuclei and many tubules contain albuminous precipitate. With a larger dose of atoxyl, however, (Dog E) a greater variety of changes is encountered. The vessels throughout are dilated and congested and there is some escape of blood into the interstitial tissue and tubules of the cortex. There is an extensive hemorrhage into the medulla, obscuring much of its structure. The glomeruli are swollen, but the glomerular tufts are irregular in size, some filling the capsular space while others occupy only a portion of it. Most of the tuft capillaries are dilated and filled with blood, and there is an albuminous precipitate in the capsular spaces. The tubular epithelium shows a peculiar series of changes. In general, it stains poorly; the cells of the convoluted tubules are swollen, granular,

ragged, and very hydropic; many have desquamated, choking the lumen of the tubule (Fig. 9). There is great irregularity in the preservation of the nuclei; many are fragmented or pyknotic, others are entirely gone. An occasional mitotic figure is seen. Practically all the tubules contain albuminous precipitate and there are a few hyaline casts. In the inner portion of the cortex there is an extreme degree of irregularity in the tubules, so that it is difficult to identify with certainty the various types. In this region the loops of Henle show a striking change; they are markedly enlarged, the cells are widely separated from one another, and are swollen and hyaline with pyknotic nuclei as shown in Fig. 9. Some of these cells are necrotic and desquamated. Another prominent feature of the atoxyl kidney is a profuse exudation of polymorphonuclear leucocytes which is most marked in the inner half of the cortex and the boundary zone.

An increase in the length of survival of the animal (Dogs C and D) gives an opportunity for the development of still further pathological changes in the kidney. In such cases, the hemorrhage invades the inner cortex as well as the medulla (Fig. 11). The tubular epithelium shows more marked disintegration of cells, many of which are hyaline while others are hydropic and swollen. There is an increase in the exudate in the tubules which consists of albuminous precipitate, colloid droplets, red blood cells, leucocytes, and casts, causing, in many instances, a marked compression of the tubular epithelium (Fig. 10). The number of casts is distinctly greater in these dogs of longer survival. In the medulla the tubular epithelium is almost completely desquamated, and the cells are intensely hyaline with pyknotic nuclei. There is a marked exudate between the tubules consisting of serum, fibrin, red blood cells, and a few leucocytes; no interstitial structures can be distinguished. Numerous mitotic figures are present in the epithelium of both cortex and medulla. Moreover, there is a very marked increase in the interstitial leucocytic exudate in these dogs of longer survival (Fig. 11).

DISCUSSION.

As far as we are able to determine from a pathological study of various arsenic kidneys, the idea that arsenical compounds as a class

produce vascular, in contradistinction to tubular, injury must be modified.

It is quite true that with a certain group of these compounds vascular lesions predominate, causing red kidneys, of which arsenious acid is the type. Even within this group of arsenicals, however, we are able to recognize certain differences in the character of the action of the different compounds. For instance, with salvarsan and neosalvarsan, hemorrhage tends to be restricted to the outer cortex or the boundary zone; the formation of hyaline thrombi in the glomerular capillaries is more pronounced; and there is a profuse albuminous exudate in the capsular space with desquamation of epithelial cells. Interstitial edema and degeneration and necrosis of the tubular epithelium are distinctly more pronounced than with arsenious acid.

As we study kidney lesions produced by other arsenicals, however, we find, as with arsacetin, that the predominating change is one of degeneration and necrosis of tubular epithelium. These pale kidneys offer a striking contrast to the red kidneys of vascular injury. Moreover, other pale kidneys, such as those produced by such substances as arsenophenylglycine and atoxyl, in which the tubular injury is the predominant feature, may show well marked vascular injury. In these cases, the hemorrhage may be zonal in character, involving much of the medulla or even the lower portion of the cortex. The tuft capillaries may be dilated and congested with more or less albuminous precipitate in the capsular space, and there may be an exudation of serum, fibrin, red blood cells, and leucocytes into the interstitial tissues.

The histological changes in the kidneys produced by a particular arsenical compound, while elastic, are quite characteristic of the action of the compound and accord with the gross appearance of the organ. Therefore, in surveying this series of arsenic kidneys from a microscopic as well as from a gross pathological point of view, we are able to differentiate two extreme types; *i. e.*, the red and the pale. The red kidney is essentially one of vascular injury, the pale kidney is predominantly one of tubular necrosis. In addition, various transitional or subgroups exist, in which the kidney, although belonging to the red type, shows relatively a great degree of

tubular injury and *vice versa.* In regard to the tubular necrosis in these kidneys, the prompt and active regeneration of the tubular epithelium following the injection of such compounds as arsacetin, arsenophenylglycine, and atoxyl, seems to preclude the possibility that the wide-spread tubular necrosis produced by these substances can be regarded as a purely anemic phenomenon. Hence, we cannot ascribe to arsenical compounds, as a class, the property of producing a purely vascular nephritis; but we must recognize the fact that arsenical compounds produce characteristic renal lesions which may be either predominantly vascular or tubular in type and that the mode of action and the character of the lesions produced are bound up with the chemical constitution of the compound.

SUMMARY.

1. We have shown that the type of renal lesion produced by compounds of arsenic varies widely: while some arsenicals produce changes in which vascular injury predominates, others produce an equally dominant tubular injury.

2. In either of these groups the character and degree of the vascular or tubular injury produced by different compounds shows further variation, such that the lesions of different arsenicals of the same group are not identical. Each compound of arsenic that we have tested, therefore, produces a lesion-complex in the kidney that is relatively characteristic for that compound.

3. The mode and character of the action of arsenicals are dependent upon the chemical constitution of the compound.

EXPLANATION OF PLATES.

The illustrations are all from untouched photomicrographs. Magnification, × 208.

PLATE 61.

FIG. 1. Arsenious acid. Dog B. Section from the outer cortex. There is marked dilatation and congestion of vessels, including the glomerular capillaries, with hemorrhage into the interstitial tissues. The tubular epithelium is swollen and degenerated.

FIG. 2. Galyl. Dog S. Section from the outer cortex. The vessels are congested and there is a slight interstitial hemorrhage. The tuft capillaries are uni-

formly dilated and contain blood and there is a slight albuminous precipitate in the capsular space. Tubular degeneration and disintegration are marked.

PLATE 62.

FIG. 3. Salvarsan. Dog Q. Section from the outer cortex. Dilatation and congestion of vessels with slight hemorrhage. The glomeruli are swollen and the tuft capillaries contain blood and numerous hyaline thrombi. There is an albuminous precipitate in the capsular space. Tubular epithelium shows degeneration and slight necrosis.

FIG. 4. Salvarsan. Dog Q. Section from the inner cortex. Marked interstitial edema. Partial disintegration of glomerular tuft and accumulation of epithelial cells and cell detritus in the capsular space. Degeneration and necrosis of tubular epithelium.

PLATE 63.

FIG. 5. Neosalvarsan. Dog R. Section from the outer cortex. The glomeruli are swollen; the tuft is compressed; the glomerular capillaries are congested and contain numerous hyaline thrombi. There is an abundant albuminous precipitate in the capsular space. Many tubules show a massive necrosis, others show degeneration and disintegration of epithelial cells with pyknotic nuclei.

PLATE 64.

FIG. 6. Arsacetin. Dog I. Section from the midcortex. Extensive necrosis *en masse* of the epithelium of the convoluted tubules with marked degeneration and slight necrosis of the loops of Henle. Vessels and glomeruli normal.

PLATE 65.

FIG. 7. Arsenophenylglycine. Dog L. Section from the outer cortex. The glomeruli are large, the tufts are compressed, and the glomerular vessels are partially collapsed. The capsular epithelium is swollen and desquamated and the capsular space is filled with necrotic cellular debris. Degeneration and necrosis of tubular epithelium are marked.

PLATE 66.

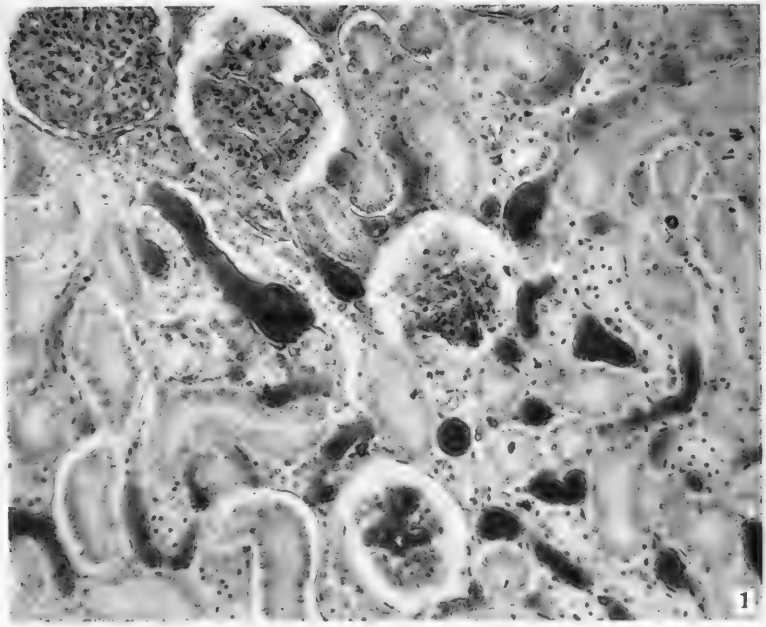
FIG. 8. Atoxyl. Dog F. Section from the outer cortex. The vessels and glomeruli are normal, except for a slight albuminous precipitate in the capsular space. The epithelium of the convoluted tubules is extremely ragged and degenerated with slight necrosis.

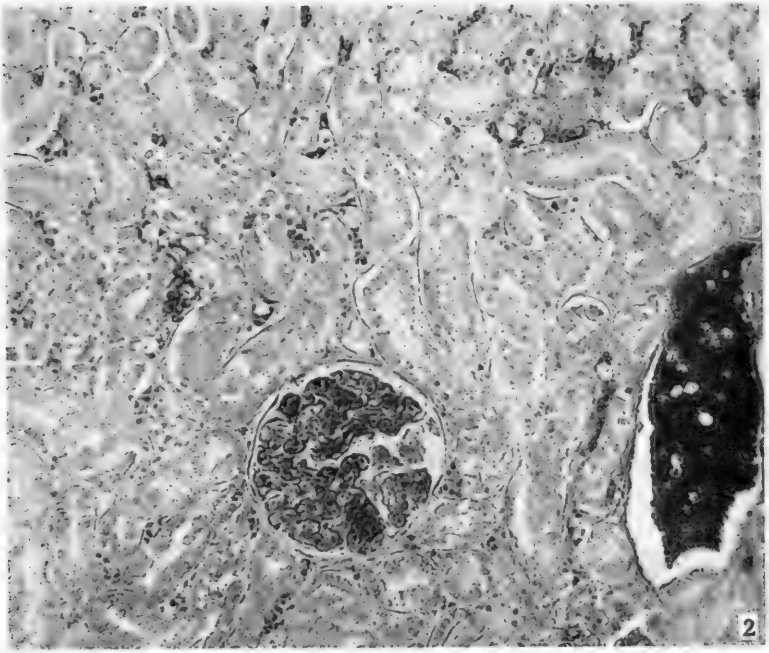
FIG. 9. Atoxyl. Dog E. Section from the midcortex. The glomeruli are slightly enlarged, the capillaries of the tuft are slightly congested, and the capsular space contains an albuminous precipitate. The epithelium of the convoluted tubules is swollen, granular, and hydropic, and in places shows necrosis. The loops of Henle are markedly dilated; the cells are hyaline with pyknotic nuclei and are widely separated from one another. Many cells are desquamated. There is a diffuse hemorrhage and polymorphonuclear exudate into the interstitial tissues and occasionally into the tubules.

PLATE 67.

FIG. 10. Atoxyl. Dog D. Section from the outer cortex. The glomeruli are enlarged. The tuft is slightly compressed and the capsular space contains an abundant albuminous precipitate. The epithelium of the convoluted tubules is extremely ragged and hydropic and in some areas the cells are necrotic and desquamated. Other tubules are filled with a granular precipitate, hyaline droplets, and an occasional cast, and the epithelium of these tubules is compressed.

FIG. 11. Atoxyl. Dog D. Section from the inner cortex. The glomeruli are as in Fig. 10. The tubules throughout are necrotic, and filled with cellular detritus or granular casts. There is a marked interstitial hemorrhage with an exudate of polymorphonuclear leucocytes.





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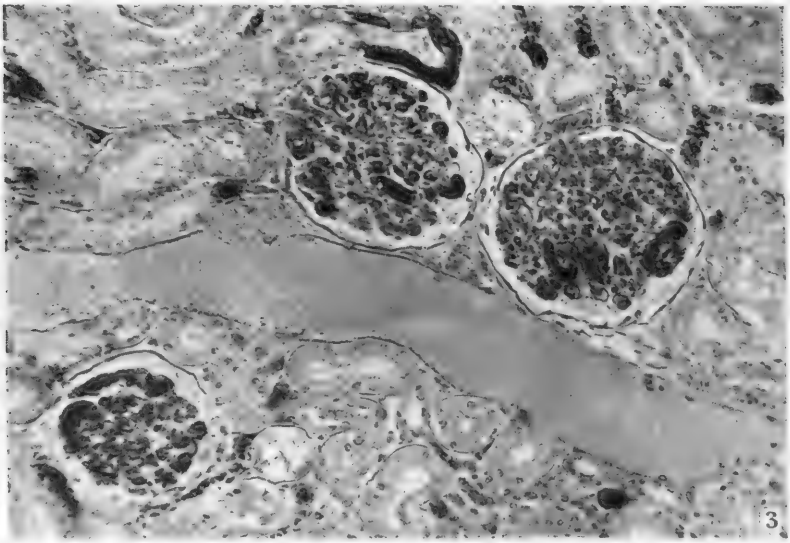

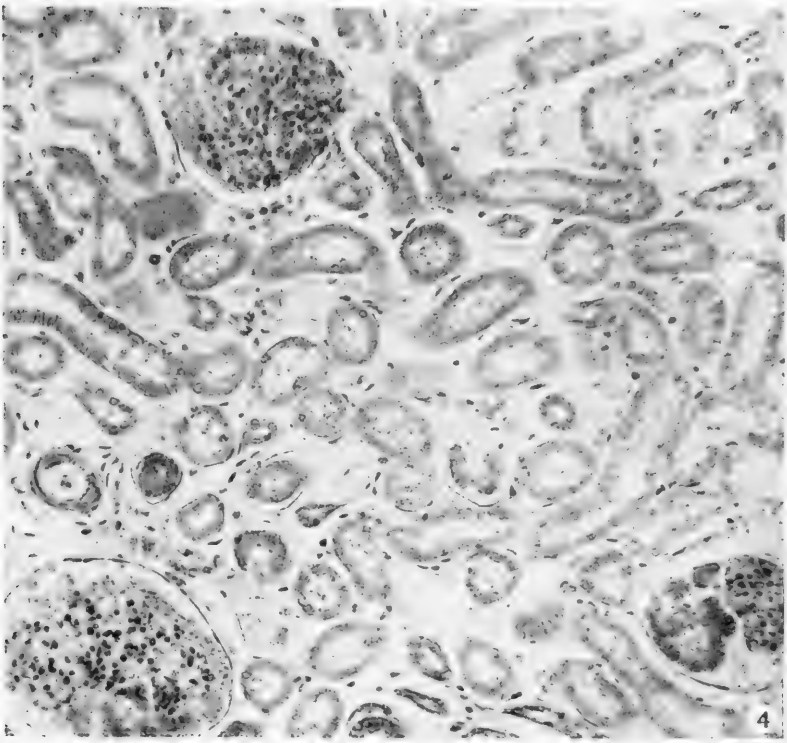

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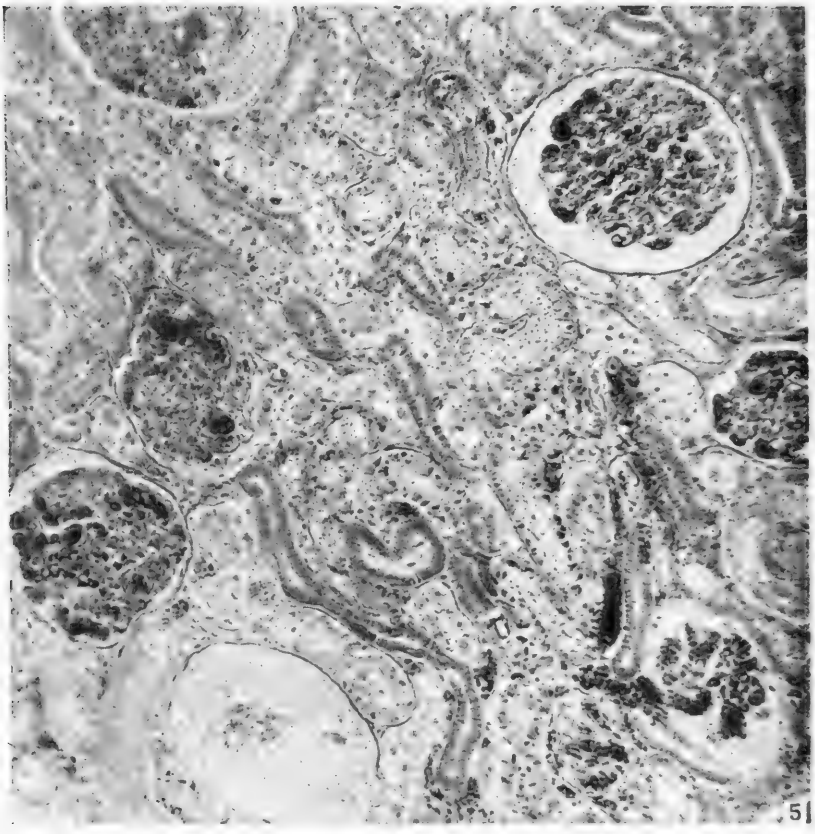

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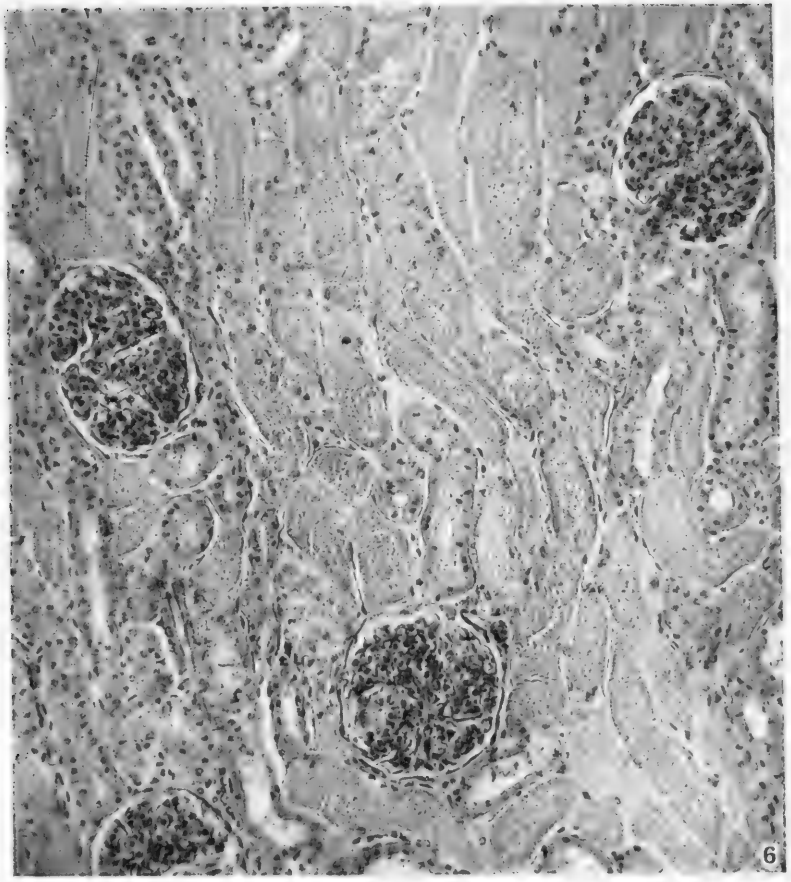

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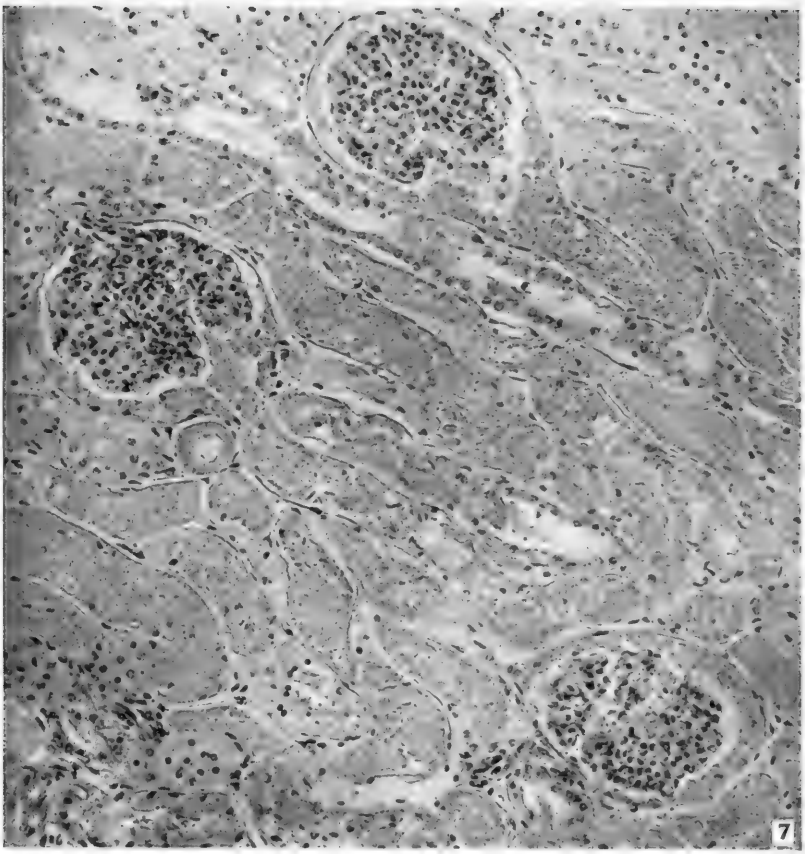

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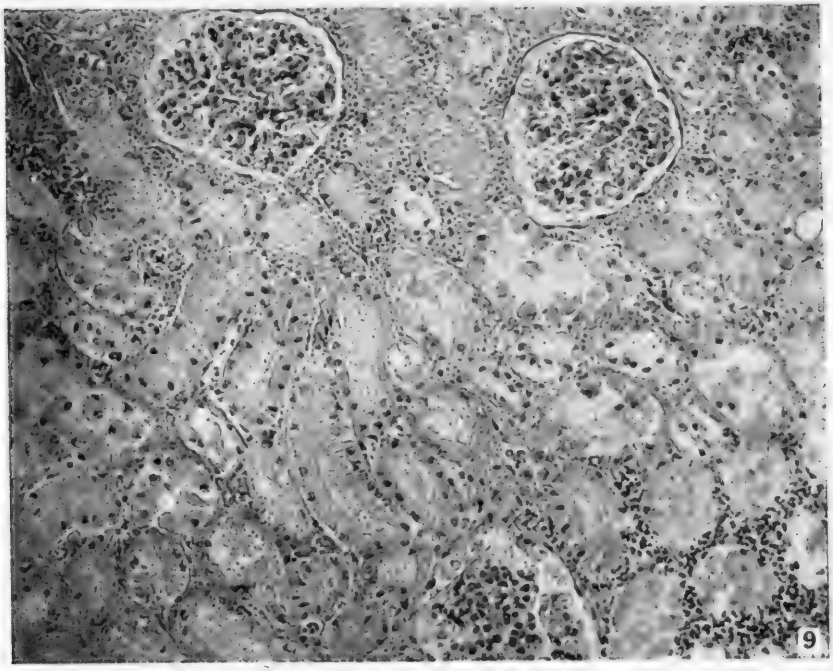

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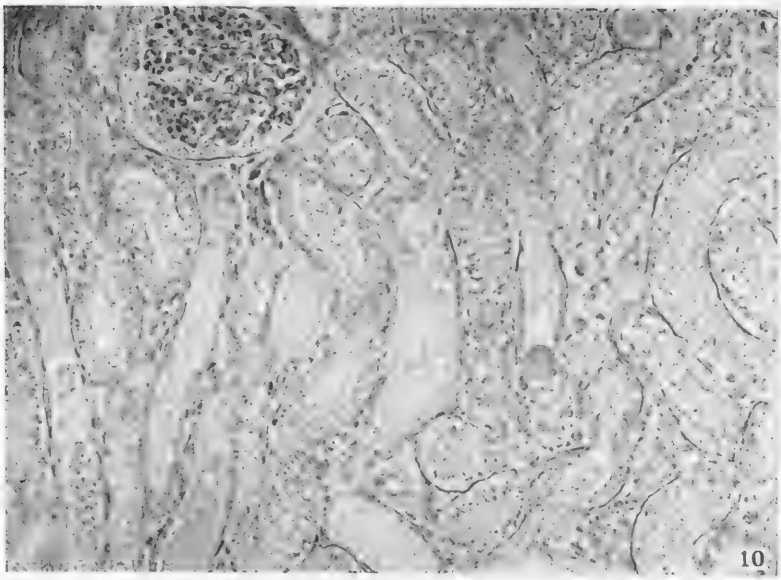

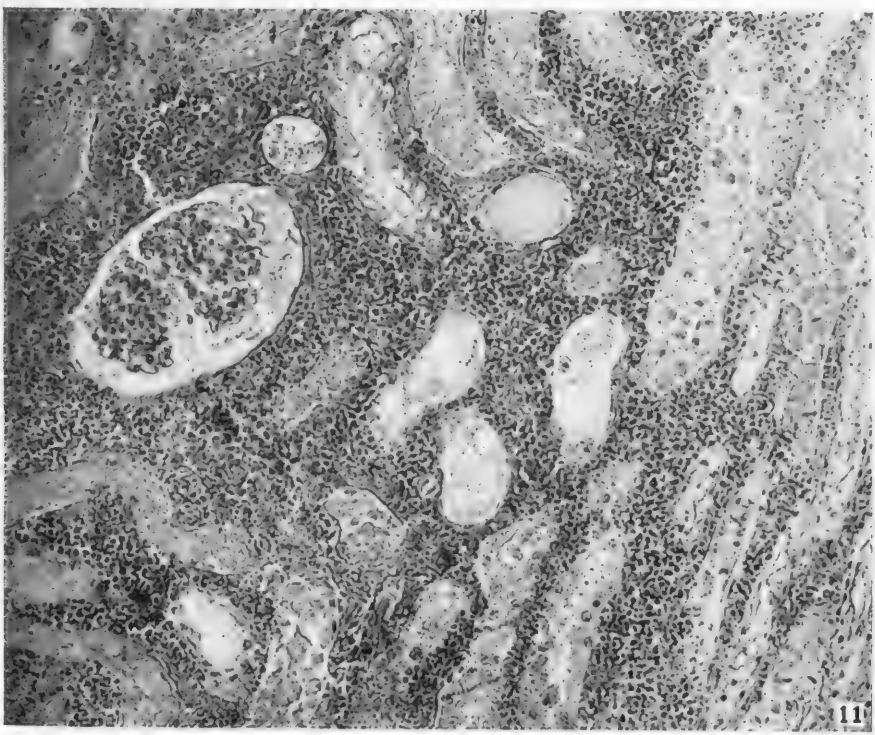

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CHEMOPATHOLOGICAL STUDIES WITH COMPOUNDS OF ARSENIC.

III. On the Pathological Action of Arsenicals on the Adrenals.

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Plates 68 to 73.

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In spite of the unusual interest that has been manifested in both the ductless glands and experimental arsenic therapy during recent years, the action of arsenicals upon the adrenals, which is one of the most constant and perhaps important features of arsenical intoxication, has remained practically unnoted.

This phase of arsenical action was first observed by us in the course of our chemotherapeutic studies. The adrenals were so constantly involved in the toxic action of our series of arsenicals that we were led to suspect that the tendency to adrenal injury was not peculiar to our compounds but was shared, to a greater or less degree, by other arsenicals. We instituted a series of experiments, therefore, to determine whether such substances as arsenious and arsenic acids, sodium cacodylate, atoxyl, arsacetin, arsenophenylglycine, salvarsan, and neosalvarsan possessed a similar tendency to adrenal injury. These substances were chosen as representative and accessible types of organic and inorganic compounds of trivalent and pentavalent arsenic.

EXPERIMENTAL.

Materials.

The routine tests of the above named drugs were made upon male guinea pigs weighing 400 to 500 grams, though supplementary evi-

dence was obtained from both rabbits and dogs which were used in order to facilitate intravenous administration of the drugs.

Technique.

Sterile solutions of the substances were injected intraperitoneally. The arsenious and arsenic acids were dissolved in the theoretical amount of sodium hydroxide and given in a 1 per cent solution. The solutions of salvarsan and neosalvarsan were prepared as for clinical use. All the other compounds were given in as dilute solution as possible, but the concentration necessarily varied somewhat with the amount of the drug given.

Dose.

On account of a lack of accurate information as to the toxic action of some of the arsenicals, we were forced to employ a wide range of doses before obtaining results that could be regarded as comparable. The following figures (Table I) were found to represent roughly the doses in grams per kilo of body weight causing death in guinea pigs in one to three days, except in the instance of arsacetin and salvarsan, where the largest doses used are stated, though they did not kill.

TABLE I.

Drug.	Dose per kilo of body weight. *gm.*
Arsenious acid	0.010 to 0.012
Arsenic acid	0.015 " 0.020
Sodium cacodylate (Merck)	0.700 " 1.000
Atoxyl	0.080 " 0.100
Arsacetin	0.300
Arsenophenylglycine	0.250 (or less)
Salvarsan	0.150
" rabbit (intravenous)	0.100 to 0.150
Neosalvarsan	0.300 " 0.350

The effects of three types of intoxication were studied with most of the compounds; *i. e.*, the effect of an acutely fatal dose, the effect of single large, sublethal doses, and the effect of repeated large doses.

Gross evidences of injury were noted, and with each animal the right adrenal was preserved in Zenker's fluid; the anterior half of the left adrenal in Müller's fluid, to preserve the chromaffin; and

the posterior half in 10 per cent formalin. Sections were always taken from corresponding levels. The Zenker and Müller material was sectioned in paraffin and stained with hematoxylin and eosin. Frozen sections were made from the formalin-fixed tissue and stained for lipoids with Herxheimer's Scharlach R counterstained with aqueous alum hematoxylin.

As the prime object of the experiments was to determine a single fact,—the presence or absence of adrenal injury as a result of arsenical intoxication—the scope of the experiments does not justify a detailed description of the action of each compound. The description given is a composite one based on the results obtained with the various arsenicals upon the adrenals of the guinea pig. Since all the compounds do not affect the adrenals in precisely the same manner, however, such peculiarities in their action as we have noted may be briefly indicated.

The Normal Adrenal of the Guinea Pig.

To facilitate description and as a basis for comparison, we wish to refer briefly to certain features of the normal adrenal of the guinea pig. The adrenals of adult male guinea pigs of the weights used show certain differences that accord in general with differences in the color of the animals. The cortex is grossly divisible into two zones: an outer waxy zone and an inner pigmented zone. In black guinea pigs the adrenals are relatively large with a narrow rim of waxy cortex sharply demarcated from a broad, intensely pigmented, inner zone. In white guinea pigs the other extreme exists; namely, small adrenals with a relatively broad rim of waxy cortex which gradually merges with a relatively narrow and slightly pigmented inner zone. These markings are doubly important since they furnish landmarks and standards for gross pathological changes in the adrenal cortex and correspond quite accurately with the normal distribution of lipoids (Figs. 1 and 2).

It so happens that the microscopic as well as the gross changes produced by arsenicals conform so closely to these divisions of the cortex that they can be most conveniently described upon such a basis of division. Without raising the question, therefore, of the relation of these zones to the generally recognized microscopic divi-

sions, the glomerulosa, the fasciculata, and the reticularis, we have used the designations, Zones 1, 2, and 3. Zone 1 corresponds with the zona glomerulosa; Zone 2 with the waxy or lipoid-containing fasciculata; and Zone 3 with the pigmented portion of the cortex which is also easily recognizable with the microscope on account of the difference in the character of the cells of Zones 2 and 3.

Gross Pathological Changes.

Large doses of all the arsenicals tested cause an acute swelling of the adrenals, usually accompanied by congestion of the surface vessels with scattered foci and streaks of hemorrhage. On section the organs are very soft; the waxy cortex appears slightly gray and translucent and may be streaked with red. Frequently a distinct red line separates the waxy and pigmented zones. The latter area is usually very soft and depressed, but rarely shows any early change in its extent or the intensity of its pigmentation. The medulla is apt to be poorly defined, soft, and somewhat congested.

Later (after forty-eight to seventy-two hours) the adrenals, though still swollen, are quite pale. On section the organ is firmer, and the waxy zone of the cortex is usually distinctly widened, gray, and translucent. The pigmented cortex is narrowed and the intensity of pigmentation is decreased. The original line of contact between these two zones is indicated by a conspicuous narrow band of opaque yellow, gray, or pink tissue. The medulla is usually normal in appearance or somewhat more conspicuous than normally.

Microscopic Changes.

Lipoids.—We have observed two types of alteration in the lipoid content of the adrenal: change in the size of the droplets and change in the amount and distribution of the lipoids. Very early, large droplets of lipoid material appear among the fine granules normally present in the cells of Zone 2 of the cortex. These large droplets are most numerous at the outer and inner edges of the zone and in the latter location, the junction of Zones 2 and 3, there appears to be an actual increase in the amount of lipoid present (Fig. 3).

Subsequently lipoid material increases in Zone 1 and begins to accumulate in considerable amounts in Zone 3 (Fig. 3), while at the

same time the amount of lipoid diminishes in Zone 2; this decrease is first apparent in the middle and inner half of the zone (Figs. 3, 4, and 5). Finally, a general depletion sets in and the lipoids disappear completely from the inner half of Zone 2, then from Zones 1 and 3, leaving only scattered droplets in the outer half of Zone 2 and possibly fine granules at the line of separation of Zones 2 and 3 (Figs. 4, 5, and 6).

Very rarely have we been able to demonstrate lipoid material in true medullary cells. In a few instances, fine granules have been observed in these cells.

Vascular Changes.—In the early stages of arsenical poisoning, the vessels of both the cortex and medulla of the adrenal are dilated and filled with blood. Hemorrhage is marked with some compounds (arsenious and arsenic acids and sodium cacodylate) especially in Zone 2 of the cortex, at the junction of Zones 2 and 3, and between the cortex and medulla (Fig. 7). Hyaline or leucocytic thrombi are occasionally seen in the vascular spaces and a slight leucocytic exudate and interstitial edema are frequently present.

Cellular and Structural Alterations.—The cortical cells of the adrenal show a variety of degenerative changes. The cells of Zone 1 are usually swollen; their cytoplasm is granular and slightly vacuolated. The cells of Zone 2 suffer most: the sharp outlines and spongy cytoplasm of these cells are soon lost; they become irregular, ragged, granular, and vacuolated; some are enormously swollen with pale-staining cytoplasm while others are shrunken. Hyaline cells are seen here and there and necrotic cells are numerous in extreme cases. All these cellular changes are again most pronounced at the junction of Zones 2 and 3 (Fig. 8). In Zone 3, cells that are usually finely granular show some small vacuoles and a coarsely granular or hyaline cytoplasm. Necrotic cells in this zone are most numerous at its inner edge.

The architecture of the cortex is frequently disturbed. The cell columns may be broken up and the cells appear completely isolated or in irregular clumps and columns.

The cells of the medulla also show evidence of injury. They are frequently ragged with scant, pale-staining, granular cytoplasm and shrunken, pyknotic nuclei. The most striking feature of the

change, with some compounds, is the presence of large numbers of colloid droplets within and among the cells of the medulla (Fig. 9). Necrotic cells may be numerous.

Chromaffin.—All the arsenicals that we have tested exercise some influence upon the chromaffin content of the adrenal. Some compounds (arsenious and arsenic acids) seem to cause but a slight reduction in the chromaffin content as judged by the color of the medullary cells of tissue fixed in Müller's fluid. Judged by the same standards, other arsenicals produce an extreme depletion of the chromaffin (sodium cacodylate, salvarsan, and neosalvarsan) as shown by comparing Figs. 10, 11, and 12.

Recovery.—The injury phase of the action of arsenicals upon the adrenals develops with a varying rapidity and persists for a variable length of time; with some compounds the changes develop rapidly and recovery is equally rapid, while with others the reverse is true. Regeneration of cortical cells is usually rapid and mitotic figures may be seen as early as twenty-four hours after the administration of the drug but are not numerous until forty-eight to seventy-two hours (Fig. 13). Here activity is most marked in the outer half of Zone 2. Six to twelve mitotic figures frequently occur in a single high power field of the microscope. Mitotic division occurs to a limited degree in the cells of Zones 1 and 3. The cells of the medulla also regenerate by mitotic division (Fig. 14). We observed mitotic figures in these cells in several instances after sodium cacodylate, atoxyl, and arsenophenylglycine, but they were never numerous.

Perhaps as a sequel to the injury produced in the medulla, round-celled or polyblastic infiltration is of frequent occurrence and is usually accompanied by some fibroblasts and an increase in endothelial cells (Figs. 15 and 16). To a less degree changes similar to those in Fig. 16 have been seen in control animals, which leaves some doubt as to the significance of this particular type of infiltration.

DISCUSSION.

Thus far, all the compounds of arsenic that we have tested have exhibited a definite action upon the adrenals of the guinea pig, but it by no means follows that the character and degree of such action

are identical for all arsenicals. On the contrary, while certain features of the action appear to be common to a number of compounds, other features of the action may be quite distinctive. Thus, arsenious acid and sodium cacodylate show a strong tendency to produce congestion and hemorrhages in the adrenals, while such compounds as arsenophenylglycine and arsacetin produce most marked disturbances in the lipoid content, with but slight tendency to congestion or hemorrhage. In like manner, arsenious acid seems to cause relatively slight alteration in the chromaffin content, while sodium cacodylate acts strongly on the medullary cells.

We must also recognize the fact that the relative importance of adrenal injury in the lesion-complex is another variable. With some arsenicals the effect upon the adrenals may be distinctly overshadowed by injury to other organs, while with other compounds the injury inflicted upon the adrenals plays a prominent part in the toxic manifestations.

We may conclude, therefore, that some factor other than the mere presence of arsenic must exercise a distinct influence upon the activity of these compounds; namely, their chemical constitution.

Finally we must differentiate between the action of toxic doses and the action of therapeutic doses of arsenicals upon the adrenals. Since the action of toxic doses indicates a strong selective affinity of the adrenals for compounds of arsenic, we may legitimately infer that with therapeutic doses injury might give place to stimulation, a conception which clinical experience with some arsenicals seems to justify.

However, generalizations as to the action of arsenicals on the adrenals should be made with caution. From these experiments concerning the action of compounds of arsenic upon the adrenals, we believe that a wide field of investigation has been opened up and that future work will justify our belief in the importance of the action of arsenicals upon the adrenals.

SUMMARY.

1. Toxic doses of all arsenicals of which we have any knowledge produce definite pathological changes in the adrenals of guinea pigs. These changes include congestion, hemorrhage, disturbances in the

lipoid content, cellular degenerations and necroses, and reduction in the chromaffin content.

2. The character and severity of the injury produced by different arsenicals varies with the chemical constitution of the compounds.

3. From these facts, we believe that adrenal injury is an important factor in arsenical intoxication and suggest that therapeutic doses of some arsenicals may produce adrenal stimulation.

EXPLANATION OF PLATES.

The illustrations are all from untouched photomicrographs. Those showing lipoids are from frozen sections stained with Herxheimer's Scharlach R counterstained with aqueous alum hematoxylin. Figures showing chromaffin are from tissue fixed in Müller's fluid and stained with hematoxylin and eosin. All doses of drugs are expressed in gm. per kilo of body weight.

Changes in the Lipoids of the Adrenal Cortex.

PLATE 68.

FIG. 1. Lipoids of normal adrenal cortex. Black male guinea pig. The lipoid, which appears as black granules, is sharply confined to the relatively narrow outer rim of the cortex. × 80.

FIG. 2. Lipoid of normal adrenal cortex. White male guinea pig. The lipoid extends over more than one-half of the cortex and is irregularly demarcated at the inner edge of the zone. × 80.

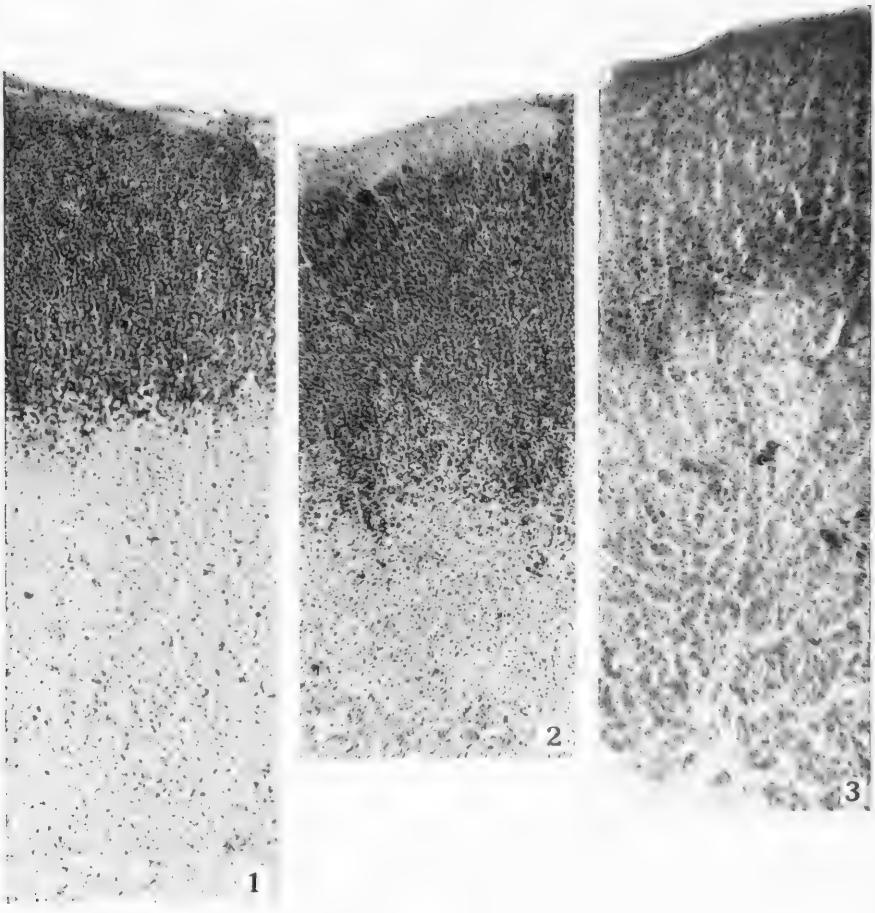
FIG. 3. Increase in the amount of demonstrable lipoid and an abnormal distribution produced by arsenophenylglycine 0.250 gm. Animal killed after 48 hours. The lipoid is distributed over the entire cortex and is especially abundant at the junction of Zones 2 and 3. The photographic intensity of the lipoid in this figure had to be considerably suppressed in the original photomicrograph in order to preserve some detail. × 80.

PLATE 69.

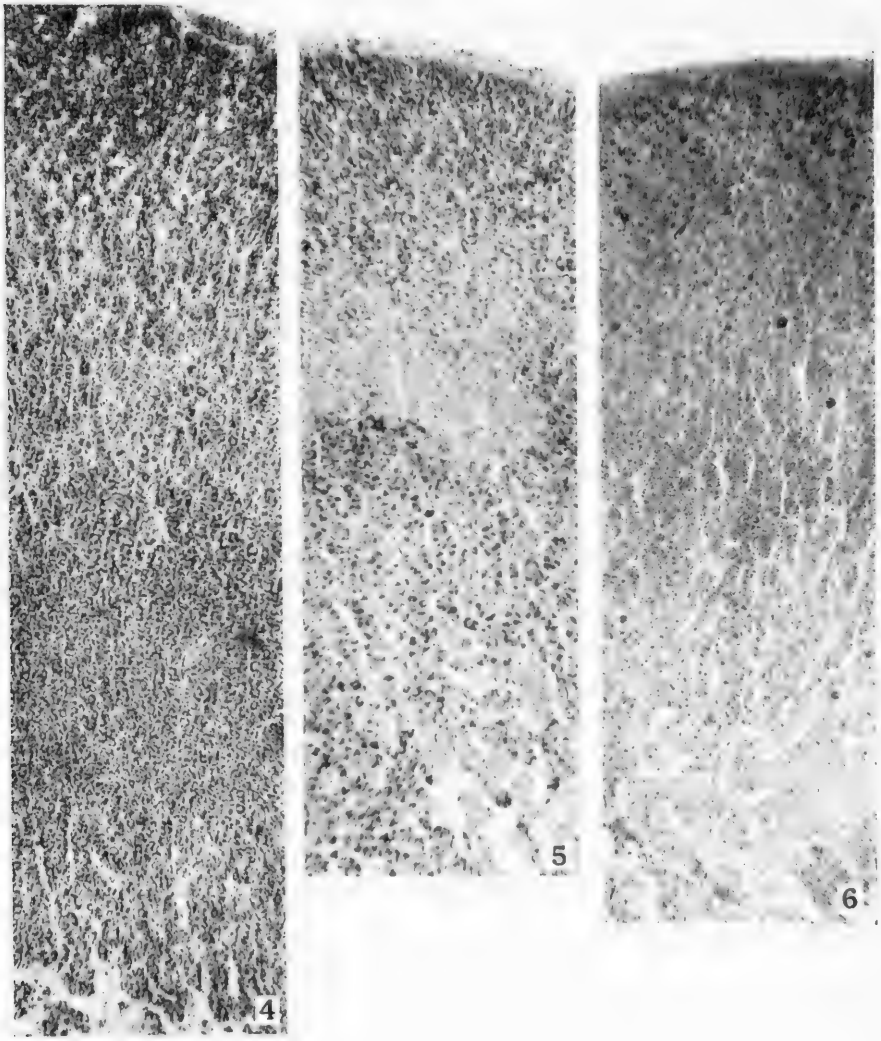
FIG. 4. A later stage of the same type of change as that in Fig. 3. A distinct decrease of the lipoids is here apparent in the inner half of Zone 2. 2 doses of arsenophenylglycine 0.100 gm., followed on the 5th day by arsenophenylglycine 0.050 gm. Animal died in 24 hours. No postmortem decomposition. × 80.

FIG. 5. Still more pronounced change in the amount, distribution, and character of the lipoid droplets. Neosalvarsan 0.300 gm. Guinea pig killed after 48 hours. × 80.

FIG. 6. Extreme depletion of lipoids produced by 4 doses of arsacetin (0.100, 0.200, 0.200, 0.200 gm.) within 16 days. Guinea pig killed 3 days after the last dose. The lipoid is mostly in the form of large droplets with some very fine granules in the cells through the middle of the cortex. × 80.



(Brown and Pearce: Action of Arsenicals on Adrenals.)



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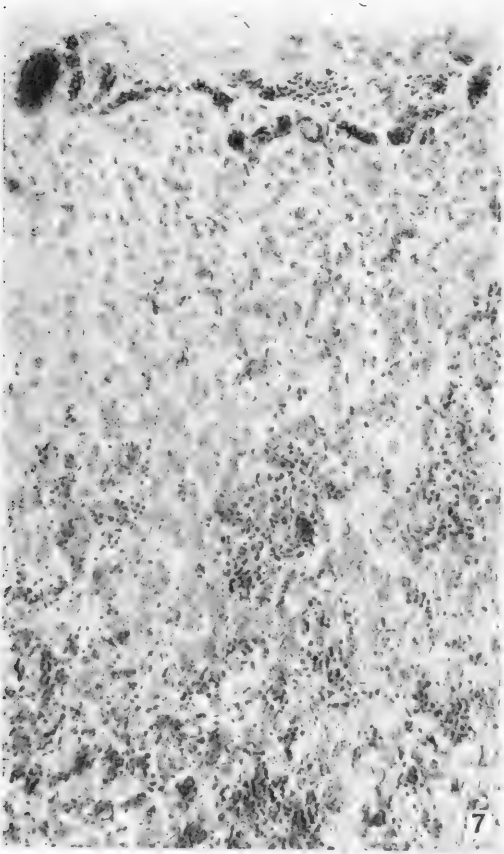

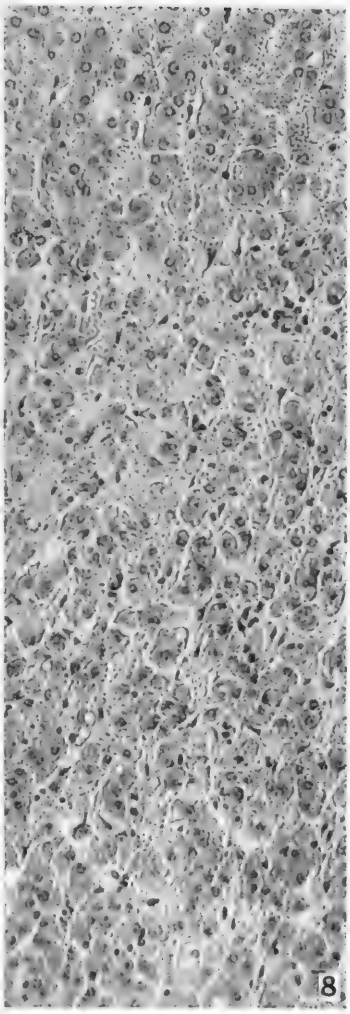

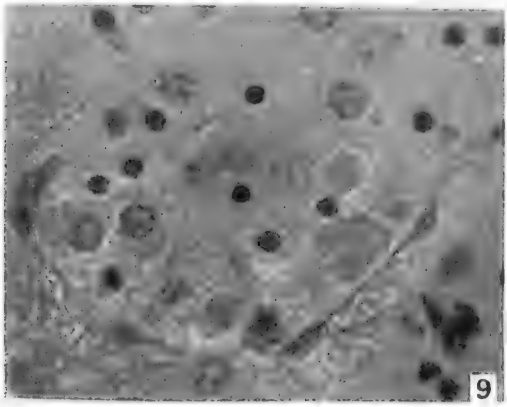

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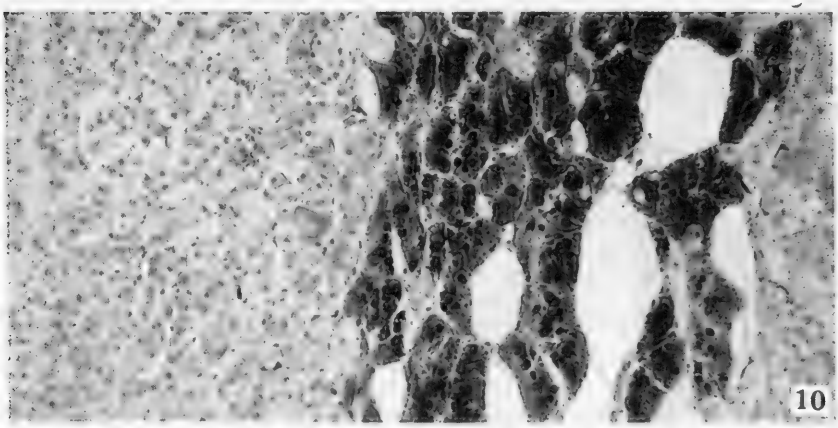
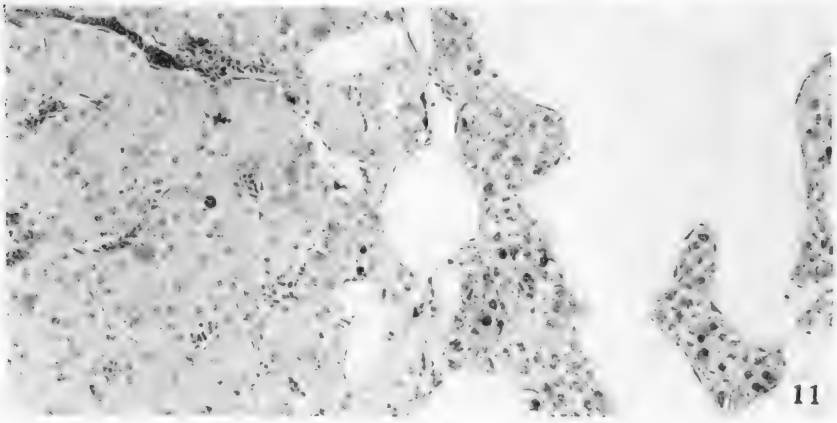
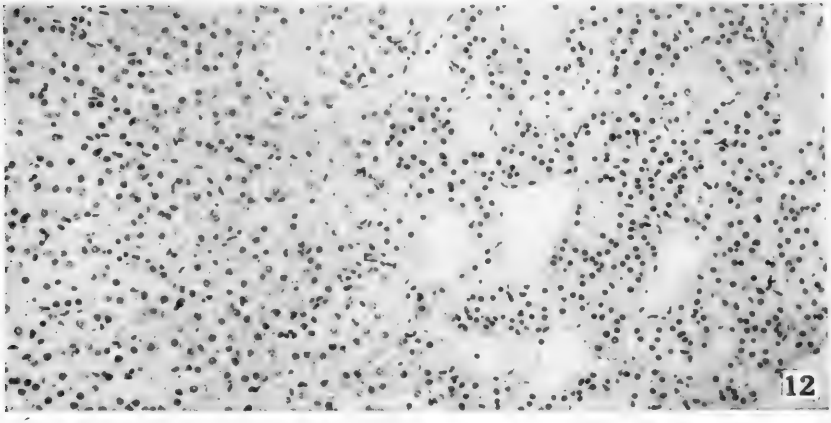
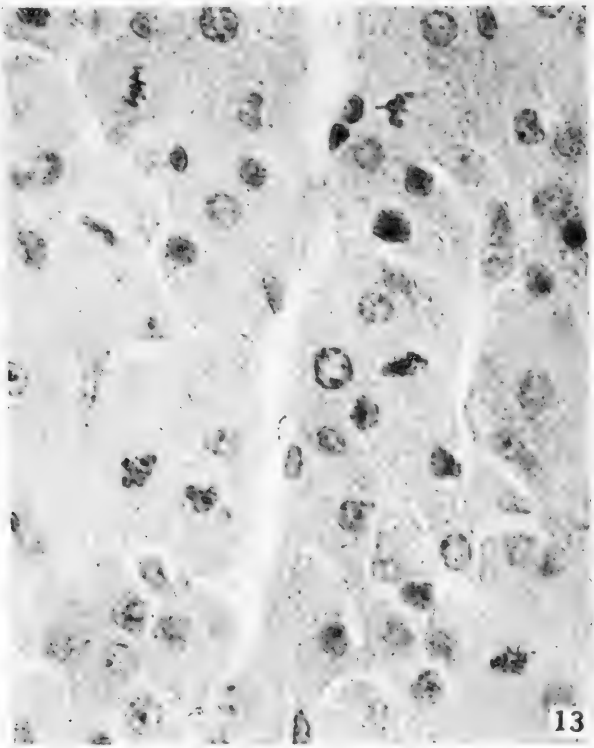
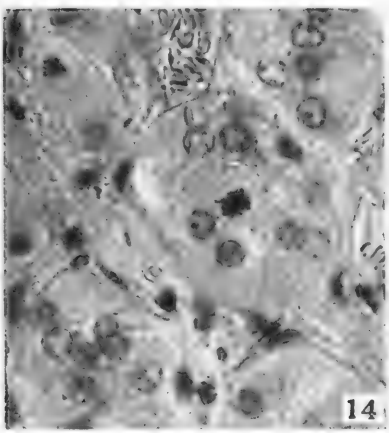






(Brown and Pearce: Action of Arsenicals on Adrenals.)





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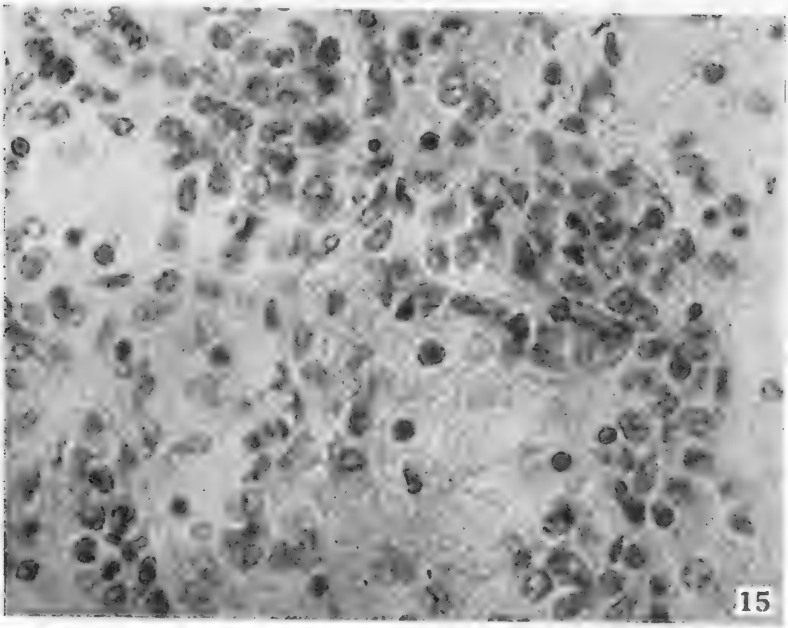

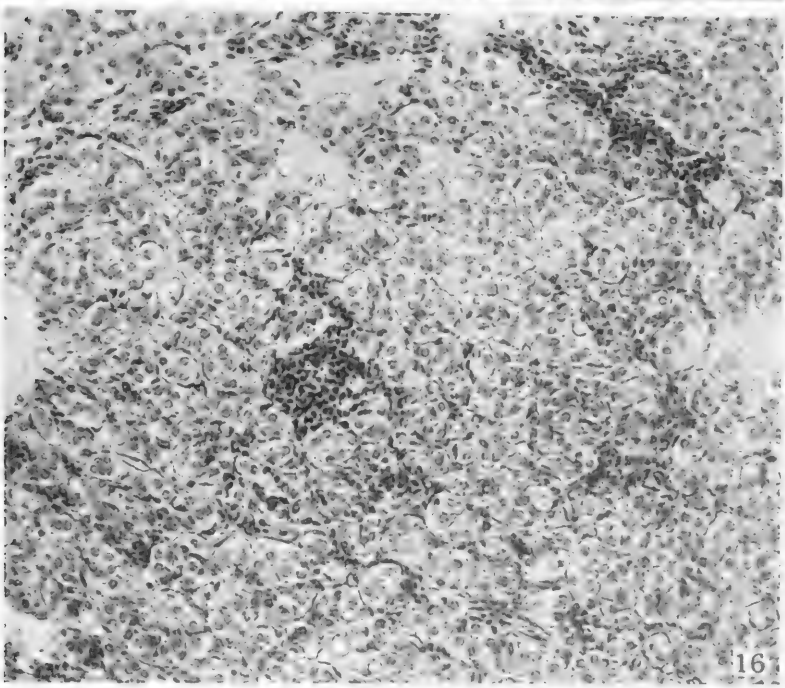

(Brown and Pearce: Action of Arsenicals on Adrenals.)

Congestion, Hemorrhage, and Degeneration.

PLATE 70.

FIG. 7. Congestion and hemorrhage in the cortex of the adrenal produced by 2 doses of sodium cacodylate 0.700 gm. in 4 days. Guinea pig died on the 5th day. Hemorrhage is most marked in the inner half of Zone 2. The cortical cells also show degeneration and vacuolization. × 200.

FIG. 8. Degeneration of the cells of the midzone of the adrenal cortex with slight leucocytic infiltration resulting from arsenophenylglycine. Same adrenal as in Fig. 4. × 200.

FIG. 9. Colloid degeneration of medulla of the adrenal. Same animal as in Figs. 4 and 8. × 775.

Changes in the Chromaffin.

PLATE 71.

FIG. 10. Chromaffin of adrenal of normal guinea pig. × 200.

FIG. 11. Reduction in the chromaffin content of the medulla with shrinkage and degeneration of medullary cells. Sodium cacodylate 0.700 gm. Guinea pig killed after 48 hours. × 200.

FIG. 12. Reduction of chromaffin after 2 doses of neosalvarsan (0.190 gm. and 0.500 gm.) on the 5th day. Died within 18 hours. No postmortem decomposition. × 200.

Regeneration of Adrenal Cells.

PLATE 72.

FIG. 13. Cortical regeneration. Neosalvarsan 0.300 gm. Killed after 48 hours. × 700.

FIG. 14. Mitosis in cell of medulla. Atoxyl, 3 doses of 0.050 gm. in 5 days. Guinea pig killed 3 days after last dose. × 600.

Infiltration in the Medulla.

PLATE 73.

FIG. 15. Infiltration of polymorphonuclear leucocytes and polyblasts into a degenerated area of the medulla. Arsenophenylglycine. Same adrenal as in Figs. 4, 8, and 9. ×700.

FIG. 16. Infiltration of lymphocytes and fibroblasts in the medulla of the adrenal. Arsenious acid 0.005 gm. Killed after 3 days.

A NOTE ON THE IMMEDIATE EFFECTS OF REDUCTION OF KIDNEY SUBSTANCE.[1]

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Several investigators have studied the nitrogenous metabolism of animals following reduction of kidney substance; notably, Tuffier,[2] Bradford,[3] Bainbridge and Beddard,[4] Pearce,[5] and Pilcher.[6] Studies of nitrogen balance and partition have been made and somewhat contradictory results obtained. de Paoli[7] observed that large quantities of kidney substance could be removed without endangering life and that the minimum necessary for life is one-half of one kidney, or one-quarter of the total kidney substance. Tuffier, on the other hand, stated that life is possible with only 1.5 gm. of kidney substance per kilo of body weight (the total is about 7 gm. per kilo in the dog). Bradford found the danger limit reached at 2 gm. per kilo; this was confirmed by Pearce and by Pilcher.

As regards function Tuffier, as quoted by Bradford, states: "It is possible to remove quantities of kidney substance equal in weight and in volume to those of the sum of the two kidneys in such a way as yet to leave behind a considerable amount of kidney substance, as shown by postmortem examination; that this diminution in the amount of kidney substance is *not* accompanied by any functional disturbance of the urine, and that the urine and urea, after oscillations due to the operation, return to their normal amount."

Bradford found that excision of less than two-thirds of the kidney volume

[1] Aided by a grant from The Rockefeller Institute for Medical Research.

[2] Tuffier, T., Études expérimentales sur la chirurgie du rein, Paris, 1889; cited by Bradford, J. R., The Results Following Partial Nephrectomy and the Influence of the Kidney on Metabolism, *Jour. Physiol.*, 1898–99, xxiii, 415.

[3] Bradford, J. R., *loc. cit.*

[4] Bainbridge, F. A., and Beddard, A. P., The Relation of the Kidneys to Metabolism, *Proc. Roy. Soc., London, Series B*, 1907, lxxix, 75.

[5] Pearce, R. M., The Influence of the Reduction of Kidney Substance upon Nitrogenous Metabolism, *Jour. Exper. Med.*, 1908, x, 632.

[6] Pilcher, J. D., On the Excretion of Nitrogen Subsequent to Ligation of Successive Branches of the Renal Arteries, *Jour. Biol. Chem.*, 1913, xiv, 389.

[7] de Paoli, E., *Centralbl. f. Chir.*, 1892, xix, 78; cited by Bradford, *loc. cit.*

resulted in temporary increase of the watery part of the urine, which became lasting when two-thirds were removed; removal of "approximately three-quarters of the total kidney weight is followed by a very great increase in the amount of urinary water, and also by an increase in the amount of urea excreted." He found also a considerable increase in the nitrogenous extractives of the blood and tissues, particularly muscles, most marked after excision of three-quarters, but quite apparent after excision of two-thirds of the kidney substance. This excess was distributed just as after double nephrectomy and after the intravenous injection of urea; he states that the quantity in the muscles is too great to be accounted for by the mere products of normal metabolism.

Bainbridge and Beddard working on cats found that removal of approximately three-fourths of the total kidney substance is not constantly followed by an increased output of nitrogen, and that it takes place only in cats which have lost 22 per cent or more of their initial body weight at the time of its onset. They concluded further that the kidneys have no direct influence upon nitrogenous metabolism and that the increased output of nitrogen is simply the result of inanition. They found the cats still able to pass a concentrated urine and that the amount of urine is not necessarily increased beyond the normal.

Pearce, using dogs, confirmed the general results of Bainbridge and Beddard and concluded further that the metabolism condition of starvation is apparently the result of the gastro-intestinal disturbance constantly associated with extensive kidney reduction and not of a disturbance of general nitrogenous metabolism. He found that the gastro-intestinal disturbance was not due to diminished absorption and found no evidence to support a theory of internal secretion on the part of the kidney.

Pilcher, using both cats and dogs, reduced the kidney substance by ligation of branches of the renal arteries. He states that with but one-fourth of the kidney substance functioning the quantity of urine was practically normal; there is marked temporary prostration, anorexia, loss of weight, and increased nitrogen output with finally a return to normal, with a slight tendency to nitrogen retention.

REPORTS OF EXPERIMENTS.

Our experiments have been conducted because of the availability of the Folin methods[8] for the determination of the non-protein nitrogen of the blood.

Dogs were kept on a constant diet and determinations were made of the total non-protein nitrogen of the blood, obtained by aseptic heart puncture. The urine was collected in the ordinary metabolism cages and the total non-protein nitrogen and urea nitrogen were determined. All analyses were made in duplicate or triplicate.

[8] Folin, O., and Denis, W., Protein Metabolism from the Standpoint of Blood and Tissue Analysis, *Jour. Biol. Chem.*, 1912, xi, 161; New Methods for the Determination of Total Non-Protein Nitrogen, Urea and Ammonia in Blood, *ibid.*, p. 527.

All the blood results are reported in mg. per 100 cc. of blood. All the urine results are reported in mg. per twenty-four hours.

Five dogs were used, but the results can be summarized in the protocols of three (Tables I, II, and III).

TABLE I.

Dog 1.

Female, Weight 6,500 Gm. Urine Negative for Albumin. Diet 200 Gm. of Dog Biscuit.

Date.	Blood. Total non-protein nitrogen	Urine. Total non-protein nitrogen.	Urine. Urea nitrogen.	Urine. Amount.
				cc.
Apr. 29	36	1,840	—	100
" 30	44	2,520	1,861	63
May 1	45	2,010	1,903	201
" 2	Left kidney (weight 17 gm.) removed under ether anesthesia			
" 3	44	1,192	859	159
	Slight diarrhea			
" 4	43	1,451	1,357	41
	Diarrhea very severe			
" 7	51	—	—	—
" 9	54	—	—	—
	Diarrhea finally cleared up. Experiment resumed			
" 24	38	—	—	—

At 12.30 p. m. the right kidney (weight 22 gm.) was removed and the animal bled at more frequent intervals, as follows:

		Total non-protein nitrogen in blood.
May 24	12.30 p. m.	Operation
	7.30 p. m.	44
" 25	1.00 a. m.	56
	1.00 p. m.	85
	7.00 p. m.	111
	11.30 p. m.	133
" 26	2.00 p. m.	196
	11.00 p. m.	227
" 27	10.00 a. m.	285
	2.00 p. m.	Animal found dead.

Autopsy shows slight superficial infection of the abdominal wound; the abdominal cavity is clean; a few cc. of gray, slightly turbid fluid are found in the left pleural cavity.

TABLE II.

Dog 2.

Female, Weight 6,000 Gm. Urine Negative for Albumin. Diet 2 1/2 Dog Biscuits.

Date.	Blood. Total non-protein nitrogen.	Urine. Total non-protein nitrogen.	Urine. Urea nitrogen.	Urine. Amount.
				cc.
Aug. 7.....	24	—	—	—
" 8.....	18	4,005	3,337	89
" 10.....	—	2,278	1,960	245
" 11.....	Left kidney (weight 35 gm.) was removed under ether anesthesia			
" 12.....	43	—	—	0
" 13.....	27	2,156	1,705	55
" 14.....	26	3,149	2,350	94
" 15.....	21	3,393	3,237	390
" 16.....	—	2,470	2,041	130
" 18.....	Right kidney was removed under ether anesthesia			

		Total non-protein nitrogen in blood.
Aug. 18.....	11.45 a. m.	Operation
	11.45 a. m.	31
	11.45 p. m.	72
Aug. 19.....	11.45 a. m.	95
	11.45 p. m.	114
" 20.....	11.45 a. m.	143
	6.00 p. m.	154
	12.00 midnight	165
" 21.....	12.00 noon	208
	10.00 p. m.	225
" 22.....	9.00 a. m.	282
	5.00 p. m.	Found dead.

Autopsy showed no sepsis or other abnormalities.

TABLE III.

Dog 3.

Female, Weight 4,000 Gm. Urine Negative for Albumin. Diet 120 Gm. Chopped Beef and 2 Dog Biscuits.

Date.	Blood. Total non-protein nitrogen.	Urine. Total non-protein nitrogen.	Urine. Urea nitrogen.	Urine. Amount.
				cc.
Aug. 24.....	23	1,823	1,568	49
" 25.....	—	3,725	3,053	192
" 28.....	Upper pole of right kidney (weight 6 gm.) was removed under ether anesthesia through lumbar incision by Dr. W. C. Quinby			
" 29.....	26	4,480	3,360	224
" 30.....	20	4,480	4,032	224
" 31.....	22	3,800	2,800	200
Sept. 1.....	Entire left kidney (weight 22 gm.) was removed under ether anesthesia			
" 2.....	28	—	—	150
" 3.....	41	4,620	3,135	330
" 4.....	35	3,995	2,820	235
" 8.....	41			
" 11.....	33 Diarrhea			
" 12.....	26 Diarrhea			
" 14.....	33 Diarrhea			
" 18.....	Remainder of right kidney (weight 17 gm.) was removed under ether anesthesia			

		Total non-protein nitrogen in blood.
Sept. 18.....	3.00 p. m.....Operation	
	3.00 p. m.....	35
" 19.....	12.00 noon.....	68
" 20.....	11.30 a. m.....	152
	5.00 p. m.....	172
" 21.....	9.30 a. m.....	227

2.00 p. m. Death occurred as the blood was being withdrawn. The last operation wound was infected; otherwise the autopsy showed nothing abnormal.

DISCUSSION OF RESULTS.

From these data it may be said that removal of approximately one-sixth of the kidney substance (Dog III) results in no marked alteration of the non-protein nitrogen of the blood or of the urine, although there is, as Pilcher expresses it, a slight tendency to accumulation[9] in the blood and increased output in the urine for a

[9] The term accumulation is used instead of retention to avoid confusion with the use of the latter term as expressing the holding of nitrogen in the body for metabolic and constructive purposes.

period of twenty-four (blood) or forty-eight hours (urine), probably to be explained by the operation. Excision of one-half the total kidney substance in the case of Dog II was followed by a distinct accumulation in the blood during the first twenty-four hours, associated, however, with anuria; such accumulation, however, did not appear in the corresponding period in Dog I, which was not anuric. As seen in both dogs, however, the output for the first forty-eight hours appears to be diminished, thus again showing a slight tendency toward accumulation for at the most forty-eight hours. The removal of approximately two-thirds of the kidney substance (Dog III) was followed by a very slight increase in output, and after twenty-four hours a slight but distinct increase in the non-protein nitrogen of the blood which lasted for three days.

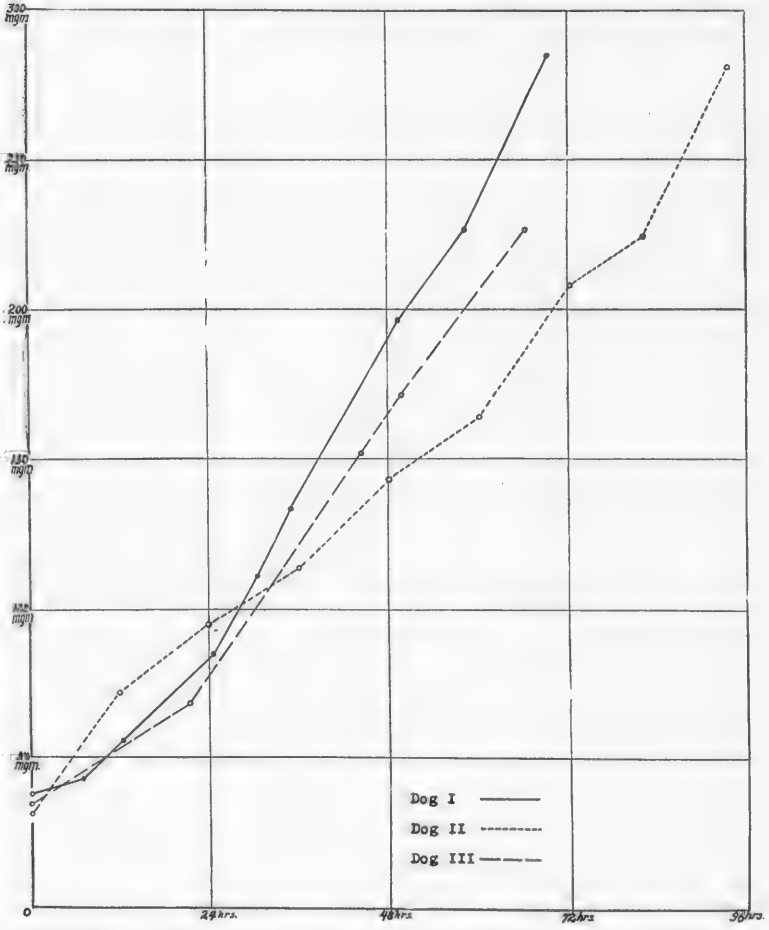
An examination of the amount of urine and its concentration shows that reduction of the kidney substance down to approximately one-third does not prevent that fragment from excreting urine in normal amounts and concentrations.

Diarrhea was found to occur frequently and followed the excision of only one-half the kidney substance. None of the dogs lost markedly in weight, and this reduction in no case reached the critical 22 per cent of Bainbridge and Beddard.

The results following the complete removal of kidney substance are shown graphically in Text-fig. 1. In Dogs I and III the rate of increase was considerably less in the first twenty-four hours than in the succeeding periods. On the second and third days the rate of increase was practically the same and from rough calculations based on the output for twenty-four hours was about what it should be per 100 cc. of blood for a twenty-four hour period. It is easily possible that operative shock might account for the relatively slight increase in the first twenty-four hours.

In Dog II the results are more constant, although in the last twenty-four hours there was a slight exacerbation of rate. In other words, for this animal the rate of increase per 100 cc. of blood should be roughly 57 mg. per day per 100 cc., so that the amounts would be for each succeeding day 88, 145, 203, and 260, whereas they actually were 95, 143, 208, and 282 (allowing twenty-one and one-half hours for the last day). It is regretted that the

actual intake of nitrogen following the total nephrectomy was not estimated and the results here presented are regarded as suggestive



TEXT-FIG. 1. Chart showing total non-protein nitrogen in blood following complete nephrectomy.

rather than conclusive. Further studies are being carried out in an attempt to elaborate on and elucidate this increase in total non-protein nitrogen in relation to intake, distribution in the body, and other factors which are known to influence protein metabolism.

A FURTHER STUDY OF THE BACTERICIDAL ACTION OF ETHYLHYDROCUPREIN ON PNEUMOCOCCI.

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In a previous communication[1] we stated that ethylhydrocuprein inhibits the growth of, and kills, pneumococci *in vitro* in very considerable dilutions of the drug, and that it exerts a considerable protective action in experimental pneumococcal infections in mice. The present study was undertaken with the object of gaining some information as to the rate of absorption of the drug into the circulation in the animal body, as to how long the resulting bactericidal effect, if any, of the serum on pneumococci lasted, and as to the mode of action of the drug on these microorganisms.

Ethylhydrocuprein, a derivative of hydroquinine, was introduced by Morgenroth[2] in 1911 in the treatment of experimental pneumococcal infection in mice. It has since been subjected to study by many observers, who, generally speaking, agree that it has a bactericidal action on pneumococci, *in vitro* and *in vivo*. Wright[3] showed that the blood serum of mice previously treated with the drug killed pneumococci in the test-tube.

In the present study we considered it advisable to make use of rabbits as our experimental animals, and, having determined the tolerated and toxic doses of the hydrochloride of the drug (optochin hydrochloride) and of the free base (optochin base) for these animals, we proceeded to study the action of each of these preparations when given by different routes as set forth below. The hydrochloride was given to the rabbits subcutaneously, dissolved in 5 cc. of

[1] Moore, H. F., *Jour. Exper. Med.*, 1915, xxii, 269.

[2] Morgenroth, J., and Levy, R., *Berl. klin. Wchnschr.*, 1911, xlviii, 1560, 1979.

[3] Wright, A. E., Morgan, W. P., Colebrook, L., and Dodgson, R. W., *Lancet*, 1912, ii, 1633, 1701.

distilled water; intravenously, dissolved in 10 cc. of physiological salt solution; and by mouth, dissolved in 25 cc. of distilled water. The free base was given subcutaneously and intramuscularly dissolved in from 5 to 6 cc. of sterile olive oil.

EXPERIMENTAL.

Our experiments show that normal rabbits of approximately 2,000 grams tolerate a single dose of 0.1 gram of the hydrochloride given subcutaneously and 0.125 gram of the base in oil given in the same manner, per kilo of body weight. It seems, however, that the tolerance of normal rabbits of greater weight—3,000 grams and upwards—is less than this. The tolerance of normal rabbits of about 2,000 grams' weight for a single dose of the base in oil when this solution is given intramuscularly (into the erector spinal mass) is still lower—0.075 gram per kilo of body weight. Finally, the animals are able to bear, without showing signs of toxicity, only a small dose of the hydrochloride dissolved in normal salt solution given slowly intravenously; namely, a dose lying between 0.02 and 0.05 of a gram per kilo of body weight. In this case the drug must be given well diluted and very slowly; for, if given in any considerable concentration, a reaction between it and the blood plasma takes place with a resulting heavy precipitate which causes speedy death.

We have observed that the tolerance of rabbits previously infected with pneumococci seems to be somewhat less than that of normal animals.

The drug in a toxic dosage gives rise to certain characteristic symptoms in rabbits, the variety and intensity of which depend in part on the dose, in part on the route by which it is given, and in part on the location of the injection. In order of severity, in the case of subcutaneous or intramuscular injection, the symptoms are as follows: quietness of the animal and disinclination to eat; halting movement of the legs nearest the side of injection; spastic and incoordinated movements of the same; and complete paralysis of the extremities nearest the injection site. Finally, the paralysis may spread to the other limbs and the animal may lie on the floor collapsed, and die. The respirations are at first hurried and, with a larger dose, later diminish in rate, and symptoms of dyspnea may

appear. When a toxic dose is given intravenously, the animal shows convulsive movements, more or less severe according to the size of the dose, and may finally die with convulsions and exophthalmos. The bactericidal action of the drug described below is seen in the serum of animals showing severe toxic signs as well as in animals which received a dose well below the toxic limit.

Technique.—Normal rabbits weighing about 2,000 grams were used. Each rabbit was bled from the marginal ear vein, the ear having previously been thoroughly cleansed with bichloride of mercury and alcohol and wiped dry with a sterile sponge. The drug was then administered in the manner and amount mentioned in each protocol below. The animals were bled either as before from the marginal ear vein, or directly from the heart—which latter may be done with safety many times—at stated intervals after the administration of the drug. The blood in each case received in centrifuge tubes was, after the final bleeding, placed in the ice chest until the following morning, when the clot was loosened and centrifugalized. 3 cc. of serum from each centrifuge tube were pipetted off the clot into a test-tube, a separate pipette being used in each case, and inactivated for one-half hour at 56° C. in a water bath. (In a few special cases the active serum was used with the object of comparing the bactericidal power of such serum with the same serum inactivated, as described above.) The 3 cc. of serum in each test-tube were inoculated with 0.5 cc. of a dilution of an 18 hour broth culture of a pneumococcus of from 1 in 100,000 to 1 in 1,000,000. Stock strains of pneumococci of Groups I and II were used, generally the latter. The pneumococci having been thoroughly mixed with the serum, 0.5 cc. of the mixture was plated in about 10 cc. of 1 per cent glucose agar, which had been previously melted and cooled to 40° C. By this means the drug, even if present in the serum in sufficient concentration to prevent the growth, or cause death, of the pneumococci, was so diluted in the plate as to leave the pneumococci free to grow unhindered. The Petri dishes used were 10 cm. in diameter. The plates were incubated for from 20 to 24 hours, at 37° C., and at the end of this period the number of colonies in each was counted. The tubes containing the inoculated serum were also incubated at 37° C. for definite periods, as stated in the

tables, at the end of each of which periods 0.5 cc. of the contents of each tube was plated as before, and these plates were incubated in the same way. In this way we were enabled to gain information on the bactericidal action on pneumococci of the serum of animals treated with ethylhydrocuprein in relation to the points already stated.

Explanation of the Protocols.—The numerals in the protocols represent the number of colonies resulting from plating 0.5 cc. of the inoculated serum from the corresponding test-tube either immediately after the tubes were inoculated, or after a definite period of incubation; the figures in the vertical columns correspond to the intervals between the administration of the drug and the various bleedings of the animal; the figures in each horizontal row represent the number of colonies per 0.5 cc. of serum from each particular bleeding after a definite period of incubation.

Experiments Illustrating the Effects on Pneumococci of the Serum of Animals Treated with Ethylhydrocuprein Hydrochloride (Optochin Hydrochloride) Dissolved in Water, Given Subcutaneously (under the Skin of the Back).

Experiment 1.—(Table I.) Rabbit 98 E; weight 1,600 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE I.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 7 hrs.' incubation.	No. of colonies when plated after 22½ hrs.' incubation.
Before giving drug.....	119	Almost infinity	Infinity.
1¼ hrs. after.....	105	36	0
4 " "	126	Almost infinity	Infinity.
5 " "	116	" "	"

Experiment 2.—(Table II.) Rabbit 115 D; weight 1,900 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE II.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 7½ hrs.' incubation.	No. of colonies when plated after 20 hrs.' incubation.
Before giving drug.....	410	Infinity	Infinity.
1⅙ hrs. after.....	445	29	3
2½ " "	492	176	Infinity.

Experiment 3.—(Table III.) Rabbit 95 E; weight 2,200 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE III.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 6½ hrs.' incubation.	No. of colonies when plated after 21 hrs.' incubation.
Before giving drug.....	329	Almost infinity	Infinity.
1 hr. after.....	268	46	18
2 " "	273	135	146
4⅚ " "	316	Several thousand	Infinity.

Experiment 4.—(Table IV.) Rabbit 141 D; weight 2,130 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I rendered highly virulent for rabbits by passage.

TABLE IV.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 6 hrs.' incubation.	No. of colonies when plated after 22 hrs.' incubation.
Before giving drug.....	281	Almost infinity	Infinity.
1⅚ hrs. after.....	351	62	0
2⅔ " "	285	134	Several thousand.
3⅔ " "	282	188	Infinity.
4⅔ " "	297	506	"
5⅚ " "	306	Almost infinity	——

Experiment 5.—(Table V.) Rabbit 2; weight 2,300 gm. Received 0.2 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. Severe toxic appearances; died immediately after last bleeding. Pneumococcus: stock strain of Group II.

TABLE V.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 2½ hrs.' incubation.	No. of colonies after 4 hrs.' incubation.	No. of colonies after 6½ hrs.' incubation.	No. of colonies after 10½ hrs.' incubation.
Before giving drug.....	456	478	Almost infinity	Infinity	—
2½ hrs. after.....	460	382	245	35	1
7⅓ " "	478	456	390	379	127

Experiment 6.—(Table VI.) Rabbit 3; weight 1,700 gm. Received 0.15 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. Severe toxic symptoms; died immediately after last bleeding. Pneumococcus: stock strain of Group II.

TABLE VI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 2 hrs.' incubation.	No. of colonies after 5 hrs.' incubation.	No. of colonies after 11 hrs.' incubation.	No. of colonies after 24 hrs.' incubation.
Before giving drug.....	187	188	Almost infinity	Infinity	Infinity.
1¼ hrs. after.....	194	127	64	0	0
2½ " "	187	175	123	56	46
4¾ " "	192	181	212	Several thousand	Infinity.

Comment on Experiments 1 to 6, Inclusive.—A study of the figures shown in the protocols of these experiments reveals certain facts. The serum of animals given a suitable dose (*e. g.,* 0.1 gram per kilo of body weight) of ethylhydrocuprein hydrochloride subcutaneously has a strongly bactericidal action *in vitro* on the pneumococci, these microorganisms growing freely in normal rabbit serum. This property is possessed by serum obtained as early as one hour after the administration of the drug—in fact, it is at its maximum about this time, or a little later; having attained a maximum potency the bacteriolytic effect gradually falls off, and, in the case of a dose of 0.1 gram per kilo of body weight (a dose well tolerated), it passes into an inhibitory effect on the growth of the pneumococci within from 2½ to 3 hours after the animal received the drug; the inhibitory effect seems to restrain the free growth of the microorganisms for some hours, after which this action seems to be overcome; this inhibitory effect on growth, in its turn, disappears about 5 hours after the drug has been given. Further, the killing off of the pneumococci in the serum of animals treated with the drug is a gradual process, lasting several hours, according to the dosage of the drug and the amount of inoculation, etc. The same effects are to be seen in the serum of animals which have received a toxic dose of the drug, except that the duration of these actions is longer, and, perhaps, more powerful.

Experiments Illustrating the Effects on Pneumococci of the Serum of Animals Treated with the Free Base Ethylhydrocuprein (Optochin Base) Dissolved in Sterile Olive Oil, Given Subcutaneously (under the Skin of the Back).

Experiment 7.—(Table VII.) Rabbit 142 D; weight 2,200 gm. Received 0.075 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I rendered highly virulent for rabbits by passage.

TABLE VII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 4 hrs.' incubation.	No. of colonies after 22 hrs.' incubation.
Before giving drug.....	301	Almost infinity	Infinity.
1½ hrs. after.....	305	116	0
2½ " "	290	227	Almost infinity.
3½ " "	297	189	" "
4½ " "	351	408	Infinity.
5 " "	358	Several thousand	——

Experiment 8.—(Table VIII.) Rabbit 91 E; weight 2,200 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE VIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.		No. of colonies after 4 hrs.' incubation.		No. of colonies after 22 hrs.' incubation.	
	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.
Before giving drug.....	321	—	Almost infinity	—	Infinity	—
1 hr. after.....	342	292	94	74	1	0
2½ " "	382	350	158	162	98	37
4¾ " "	305	323	207	257	Several thousand	Almost infinity.

Experiment 9.—(Table IX.) Rabbit 105 D; weight 1,750 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I.

TABLE IX.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 7½ hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	573	Several thousand	Infinity	—
2 hrs. after.....	349	200	41	0
3 " "	402	224	76	3
4⅙ " "	400	270	123	17
5⅙ " "	410	238	138	8

Experiment 10.—(Table X.) Rabbit 106 D; weight 1,700 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I.

TABLE X.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 7½ hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	397	Several thousand	Infinity	—
2 hrs. after.....	330	197	59	0
3 " "	338	229	125	14
4⅙ " "	358	298	190	552*
5⅙ " "	374	460	—	—

* Macroscopically there appeared to be no growth in the corresponding tube.

Experiment 11.—(Table XI.) Rabbit 104 D; weight 1,650 gm. Received 0.125 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. Animal showed a tendency to lie quiet for some hours after the injection. Pneumococcus: stock strain of Group I.

TABLE XI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 7½ hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	594	Several thousand	Infinity	Infinity.
2 hrs. after.....	520	149	51	0
3½ " "	419	159	107	0
4½ " "	368	256	1,200	Infinity.

Experiment 12.—(Table XII.) Rabbit 101 D; weight 1,720 gm. Received 0.15 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. Animal apparently well up to 6 hours after injection; died during the last bleeding. Pneumococcus: stock strain of Group II.

TABLE XII.

Serum obtained.	No. of colonies per 0.5 c. when plated immediately after inoculation.	No. of colonies after 5½ hrs.' incubation.	No. of colonies after 8½ hrs.' incubation.	No. of colonies after 25 hrs.' incubation.
Before giving drug...	976	Almost infinity	Infinity	Infinity.
2 hrs. after.....	1,100	180	57	0
3½ " "	1,328	386	292	0
5 " "	1,152	403	283	Almost infinity.
6 " "	1,284	Several thousand	Infinity	Infinity.

Experiment 13.—(Table XIII.) Rabbit 102 D; weight 1,770 gm. Received 0.15 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 5½ hrs.' incubation.	No. of colonies after 8½ hrs.' incubation.	No. of colonies after 25 hrs.' incubation.
Before giving drug...	1,176	Infinity	Infinity	Infinity.
2 hrs. after.....	1,232	452	224	10
3½ " "	958	339	169	Infinity.
5 " "	1,152	500	441	Almost infinity.

Experiment 14.—(Table XIV.) Rabbit 121 F; weight 2,050 gm. Received 0.15 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XIV.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 5¼ hrs.' incubation.	No. of colonies after 23 hrs.' incubation.
Before giving drug.....	68	Almost infinity	Infinity.
2 hrs. after.....	58	0	0
3 " "	64	0	0
4 " "	59	7	0
5 " "	43	18	0

Comment on Experiments 7 to 14, Inclusive.—These protocols, like those of Experiments 1 to 6, show that the serum of animals given subcutaneously a suitable dose of ethylhydrocuprein base (optochin base) dissolved in olive oil has a strongly bactericidal action *in vitro* on the pneumococci, that this property is possessed by serum obtained as early as 1 hour after the administration of the drug, and

that having attained a maximum potency the bacteriologic effect gradually falls off. In the case of a dose of 0.1 gram per kilo of body weight (a dose well tolerated), the bactericidal action passes into an inhibitory effect on the growth of the pneumococci in about four hours after the animal received the drug; here again, the inhibitory effect seems to restrain the free growth of the microorganism for some hours, after which this action seems to be overcome. The inhibitory effect, in its turn, ultimately disappears. As before, the protocols show that the killing off of the pneumococci in the serum of the treated animals is a gradual process. The effects are more lasting than when the hydrochloride in water is given by the same route.

Experiments Showing the Effects on Pneumococci of the Serum of Animals Treated with Ethylhydrocuprein Hydrochloride Dissolved in 10 Cc. of Normal Saline Solution and Given Slowly Intravenously.

Experiment 15.—(Table XV.) Rabbit 9; weight 2,000 gm. Received 0.02 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 10 cc. of normal saline, slowly, intravenously.

Slight convulsions toward end of injection; immediately after injection the animal lay stretched out on floor and apparently could not rise. Complete recovery in a few minutes. Pneumococcus: stock strain of Group II.

TABLE XV.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 7 hrs.' incubation.	No. of colonies after 18 hrs.' incubation.
Before giving drug.....	405	Infinity	Infinity.
5 min. after.....	426	533	About 2,000.
1½ hrs. "	360	Infinity	Infinity.

Experiment 16.—(Table XVI.) Rabbit 10; weight 2,700 gm. Received 0.078 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 10 cc. of normal saline, slowly, intravenously.

Convulsions and exophthalmos toward the end of the injection; immediately after injection the animal lay on floor and seemed collapsed; twitching of legs. Recovered in a few minutes. Pneumococcus: stock strain of Group II.

TABLE XVI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3½ hrs.' incubation.	No. of colonies after 7 hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	304	249	Infinity	Infinity.
5 min. after.....	329	235	87	10
1½ hrs. "	340	315	Infinity	—
4½ " "	350	292	"	—

The serum of rabbits which had been given intravenously doses of the hydrochloride smaller than those given to Rabbits 9 and 10 showed no bactericidal or inhibitory effect on the pneumococci. An animal given 0.05 gm. per kilo of body weight intravenously died immediately after the injection with convulsions.

Comment on Experiments 15 and 16.—The serum of rabbits given a non-fatal dose of hydrochloride of the drug intravenously does not show such a strong or prolonged effect on pneumococci as when the drug is given subcutaneously. When given intravenously, toxic signs are more easily obtained than when other routes are used.

Experiments Showing the Effects on Pneumococci of the Serum of Animals Treated with Ethylhydrocuprein Base (Optochin Base), Dissolved in Olive Oil, and Given Intramuscularly.

Experiment 17.—(Table XVII.) Rabbit 120 D; weight 1,620 gm. Received 0.075 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, intramuscularly. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XVII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 10 hrs.' incubation.	No. of colonies after 22 hrs. incubation.
Before giving drug.....	61	Infinity	Infinity.
1½ hrs. after.....	60	142	"
3½ " "	71	Almost infinity	"
4½ " "	77	Infinity	"
4⅚ " "	73	"	"

Experiment 18.—(Table XVIII.) Rabbit 121 D; weight 1,850 gm. Received 0.075 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, intramuscularly. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XVIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 10 hrs.' incubation.	No. of colonies after 22 hrs.' incubation.
Before giving drug.....	74	Infinity	Infinity.
1½ hrs. after.....	64	15	2
3½ " "	91	Several thousand	Infinity.
4¼ " "	91	Almost infinity	"
5 " "	89	Infinity	"

Experiment 19.—(Table XIX.) Rabbit 169 D; weight 2,000 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, intramuscularly. Distinct toxic symptoms within 2 hours after administration of drug. Recovered next day. Pneumococcus: stock strain of Group II.

TABLE XIX.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 4½ hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug.....	1,012	Almost infinity	Infinity.
1½ hrs. after.....	984	124	1
2½ " "	952	413	Infinity.
4½ " "	1,010	Several thousand	"

Comment on Experiments 17 to 19, Inclusive.—These experiments, illustrating the results of giving the base of the drug dissolved in oil intramuscularly, show that the drug is more toxic when given by this route than when given subcutaneously in the same vehicle and that the bactericidal effect on the pneumococci is not so prolonged, and probably not so intense, as when the subcutaneous route is used.

In addition to the experiments mentioned above, we have studied the bactericidal action of the serum of rabbits into the stomachs of which the hydrochloride of the drug, dissolved in 25 cc. of distilled water, was introduced by means of a stomach tube; the tolerance is greatest by this route, but the bactericidal effects are slight or absent; even by giving doses as large as 0.3 to 0.4 gram per kilo of body weight to rabbits of 2,000 grams' weight, and otherwise using the same technique as described above, we have not been able to demonstrate any bactericidal action of the serum of these animals on pneumococci within a period of from 2½ to 6 hours after administration of the drug, and only a slight inhibitory effect on their growth.

In order to gain some idea of the bactericidal action on pneumococci of the serum of man treated with the drug the experiments immediately to be described were carried out. The technique was the same as that described above.

Experiment 20.—(Table XX.) Normal man, M.; weight 50.8 kilos. Received, *per os,* in capsules, 0.5 gm. of ethylhydrocuprein hydrochloride. No toxic signs or symptoms. Pneumococcus: stock strain of Group II.

TABLE XX.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 2½ hrs.' incubation.	No. of colonies after 8 hrs.' incubation.	No. of colonies after 12 hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug..	395	3,000 (approximately)	Infinity	Infinity	—
1 hr. after.....	458	382	391	1,500	Several thousand.
2 " "	560	311	337	3,000 (approximately)	Several thousand.
5 " "	374	400	Several thousand	Almost infinity	Almost infinity.

Experiment 21.—(Table XXI.) Normal man, A; weight 68.96 kilos. Received *per os,* in capsules, 0.5 gm. of ethylhydrocuprein hydrochloride. No toxic signs or symptoms. Pneumococcus: stock strain of Group II.

TABLE XXI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 4⅔ hrs.' incubation.	No. of colonies after 9⅔ hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug...	517	Almost infinity	Infinity	Infinity.
1½ hrs. after.....	560	540	Almost infinity	"
2½ " "	521	230	225	Almost infinity.
5⅔ " "	518	483	Several thousand	Infinity.

Experiment 22.—(Table XXII.) Man, B.; weight 52 kilos. Received 0.5 gm. of ethylhydrocuprein hydrochloride dissolved in 5.0 cc. of sterile redistilled water, subcutaneously, in left flank. Next day complained of pains in legs, had a slight rise of temperature, and the area around the injection site was hyperemic and showed a slight superficial edema. Pneumococcus: stock strain of Group II.

TABLE XXII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.		No. of colonies after 3 hrs.' incubation.		No. of colonies after 8⅔ hrs.' incubation.		No. of colonies after 21 hrs.' incubation.	
	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.
Before giving drug....	76	112	1,300	2,000	Almost infinity	Infinity	Infinity	Infinity.
1 hr. after.....	68	54	25	40	34	19	Almost infinity	Infinity.
2 " "	65	52	29	27	22	140	Infinity	Infinity.
3 " "	61	68	38	37	153	467	"	Infinity.
4½ " "	56	59	24	31	713	431	"	Infinity.

Experiment 23.—(Table XXIII.) Woman, S.; weight 51 kilos. Received 0.5 gm. of ethylhydrocuprein hydrochloride dissolved in 5 cc. of sterile redistilled water, subcutaneously, in abdominal wall. Next day the patient complained of general malaise and headache, had a slight rise of temperature, and had a painful hyperemic area of infiltration and edema about 3 inches in diameter around the injection site which disappeared in a few days. Pneumococcus: stock strain of Group II.

TABLE XXIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 8⅔ hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug.....	60	51	Almost infinity	Infinity.
1 hr. after.....	56	35	1,320	"
2½ " "	74	34	439	"

Comment on Experiments 20 to 23, Inclusive.—The figures show that the serum of a man to whom had been given a single dose of 0.5 gram of the hydrochloride of the drug either by the mouth or subcutaneously, has a decided inhibitory effect on the growth of the pneumococcus lasting for several hours, after which this action seems to be overcome, and has a bactericidal action which is much less strong than that shown in the protocols of the animal experiments described above. Similar effects are seen whether the drug be administered subcutaneously or by mouth. The employment of the

former method, however, subjects the patient to considerable discomfort.

The serum of a normal man to whom 1.8 grams of the hydrochloride of the drug were given by the mouth in 24 hours, that is, in four doses of 0.45 gram each given at regular intervals, showed a cumulative effect; namely, the bactericidal action was much stronger 2¾ hours after the last, than at a similar period after the first, dose.

DISCUSSION OF RESULTS.

In serum, the pneumococci show a tendency to form short chains composed of from two to four diploid forms. The figures in the foregoing protocols consequently must be regarded, not as giving the total number of pneumococci per 0.5 cc. of the inoculated serum, but as an index to that number. The figures, in other words, are relative, not absolute, but the conclusions drawn with regard to the bactericidal action of ethylhydrocuprein are perfectly valid, for the growth in normal serum serves as a control. Moreover, we have cumulative evidence of this bactericidal action of the drug in the fact that the progressive diminution in the number of pneumococci is seen in a considerable number of different experiments. Again, this progressive diminution frequently goes to the point of complete disappearance of living pneumococci in a few hours.

The protocols, therefore, set forth above show that the serum of rabbits treated with ethylhydrocuprein exerts a bactericidal action on the pneumococci in the test-tube, and later inhibits their growth. The intensity and the time of appearance and disappearance of these actions depend on the dosage, upon the route by which the drug is given, and on the method of administration. With the dosage used in the experiments above described, these effects last longest when the base is given in oil subcutaneously; they are not quite so lasting when the hydrochloride is given in water, subcutaneously; and are still less lasting when the base is given in oil, intramuscularly. To obtain bactericidal effects in rabbits by giving the hydrochloride dissolved in physiological saline intravenously, one must give toxic doses dangerous to the life of the animal. The giving of the hydrochloride directly into the stomach of rabbits does not seem to be efficient from the point of view of a resulting bactericidal action of the serum on the pneumococci.

It was thought that a possible explanation of the progressive decrease in the number of colonies in those tubes in which this was demonstrated might be an agglutinative effect of the drug on the pneumococci. We have frequently examined the contents of the tubes microscopically, macroscopically, and by cultural methods for sterility (the latter in the cases in which the plates showed no colonies), and we have never found any evidences of agglutination.

It will be noticed in the protocols of several experiments that, in the case of serum obtained when, apparently, the optochin concentration in the blood is diminishing, the number of colonies in the incubated tubes at first shows a progressive decrease (the number in the control normal serum, obtained before giving the drug, showing, at the same time, a progressive increase); but, that, later, after a few hours' incubation, the pneumococci progressively increase, until they become too numerous to count in the plates. This phenomenon is more apparent in tubes lightly inoculated than in tubes more heavily inoculated with pneumococci. Evidently, if the optochin concentration in the serum falls below a certain point in relation to the number of pneumococci present, the pneumococci which survive the bactericidal action may, after a few hours in contact with the drug in the incubator, acquire the property of overcoming this action of the drug and, therefore, grow freely. It would be interesting to investigate this property, so rapidly acquired, from the point of view of fastness. It is not due to a destruction of the bactericidal action of the serum caused by simple incubation, because serum incubated for 24 hours, and then inoculated and studied as above described, shows the same intensity of bactericidal action on pneumococci as samples of the same serum not previously subjected to incubation before inoculation. This overcoming of the action of the drug seems to be an entirely new, and, relatively, quickly acquired property of the pneumococci themselves.

It does not seem that there is any considerable difference in the bacteriolytic or inhibitory effects of serum obtained after administration of the drug whether such serum be used after having been inactivated for ½ hour at 56° C., or active.

CONCLUSIONS.

1. The serum of rabbits which have been previously treated with a single dose of ethylhydrocuprein (optochin) exerts a bactericidal action on, and, later, inhibits the growth of pneumococci in the test-tube.

2. These actions are most evident in the serum of rabbits when the base (optochin base) is given in oil subcutaneously; somewhat less when the hydrochloride of the drug is given in water subcutaneously; slight when the base is given in oil intramuscularly; and least evident, or absent, when the hydrochloride in water is introduced directly into the stomach. To get these effects by the intravenous route, toxic doses must be given, and, even with toxic non-fatal doses, the effects do not last long.

3. In the case of the base given in oil subcutaneously to rabbits in a dosage of 0.1 gram per kilo of body weight, the bactericidal action of the serum is at its maximum about one hour after administration, and it passes into an inhibitory effect about four hours after the drug has been given.

4. In man the same inhibitory and bactericidal actions of the serum are present when a single dose of 0.5 gram of the hydrochloride of the drug is given by the mouth or subcutaneously, but the bactericidal action is not so marked as in rabbits.

5. When the optochin concentration in the serum has, apparently, diminished to a certain point in relation to the number of pneumococci present, the pneumococci which have survived the bactericidal action for a few hours acquire the power of growing freely.

THE SERUM FERMENTS AND ANTIFERMENT DURING PNEUMONIA.

Studies on Ferment Action. XXIV.

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Our knowledge concerning the factors that induce the crisis in pneumonia is still unsatisfactory, despite the innumerable attempts which have been made in the endeavor to advance our understanding of the processes by various immunological methods of investigation. There have been demonstrated only slight changes in the serum complement and serum antibodies before or after crisis; the opsonic and leucocyte activity, while increased, shows no striking difference; the infecting organism may show no change in its virulence or its resistance (Rosenow); and yet in a period of a few hours a phenomenon occurs, which in its sharp demarcation more nearly resembles a chemical reaction *in vitro* than a biological process *in vivo*. In so far as we may consider the involved tissue as being isolated from the general circulation, as Kline and Winternitz (1) have recently pointed out, the process must of necessity be largely local in its origin and effect. Recovery is coincident with the destruction not only of bacteria, but also with the removal of the great mass of fibrinous and cellular detritus. It is, therefore, in all of its essentials an autolytic process, and our hope of therapeutic results must be based not only on the idea of overcoming the infecting organism but also of favorably influencing the autolytic changes. The isolation of the lung tissue from the general circulation favors autolysis, for if the tissue were freely supplied with blood serum, with its great concentration of antiferment, no autolysis could take place.

Inception of Autolysis.

In a general way we can consider the factors that influence the inception of autolysis as depending on a balance between the amount of ferment, on the one hand, and the inhibiting elements, on the other, both of which are variables and subject to changes either through chemical or physical alteration. With the disintegration of the accumulated polymorphonuclear leucocytes, a large amount of a powerful polyvalent ferment is liberated, capable of dissolving the fibrin and cellular debris. This ferment is not active in the presence of blood serum because of the excess of antiferment. This inhibiting factor can be influenced either by a change in dispersion, whereby the unsaturated lipoids are rendered less disperse, *i. e.*, when the reaction becomes acid (Opie (2)); by an increased oxidation, as, for example, by iodine (3), whereby the unsaturated bonds of the antiferment are reduced and the antitryptic activity is lessened; by an increased metabolic demand on the lipoids, as, for instance, during starvation, when a marked fall in antiferment occurs (4); and by a saturation with an excess of ferment. During the course of the pneumonia the leucocytes are undergoing disintegration with a resulting liberation of ferment. The blood serum, on the other hand, is subject to various of the above mentioned factors which tend to lower the antiferment power (after the original toxic rise has occurred). We should therefore expect that at some moment the balance would be destroyed and autolysis actively commence. Almagià (5) has recently studied this relation and has called attention to the coincidence of evidences of autolysis with the crisis. He considers the very production of an autolyzing fluid as the factor which inhibits the further multiplication of the pneumococci, for he found that the growth of pneumococci was completely inhibited by autolyzing fibrin. We can thus readily see that the pneumonic process, apart from complications, is actually a self-limited one, for even if the organism is so virulent that it will readily kill the leucocytes this very property will set free a large amount of ferment in a short space of time and thus will shorten the course of the disease.

We must, however, take cognizance of another possible factor; *i. e.*, the question whether or not the blood serum itself may contain an increased amount of ferment derived from the tissue cells of the

body, which, mobilized, would be brought to bear on the diseased organ. That during the course of a pneumonia serum ferments may be found which digest lung tissue and placenta has repeatedly been noted.[1] These ferments are not specific; experiments which might be so interpreted are based on the fact that ordinary immunity reactions occur which by means of adsorption phenomena permit proteolysis of the serum proteins. Guggenheimer (6) has studied the problem in another way, in that he has determined the inhibitory or accelerating effect of the pneumonic serum on liver autolysis. He noted that pneumonic sera occasionally acted in an auxiliary way and regards this as evidence of a mobilization of cellular ferments. Other sera, however, showed a marked inhibiting effect, as did the normal serum.

Early during the course of antiferment investigations Ascoli and Bezzola (7) studied the conditions obtaining during pneumonia. With the Gross-Fuld method they noted an early rise in antiferment titer, with a decline after the crisis.

The serum investigations so far reported have lacked any quantitative basis, because the methods used to determine the amount of ferment and antiferment are largely qualitative. In order to obtain a more definite insight into the ferment-antiferment balance of the serum we have undertaken a study of a number of patients during the course of pneumonia.

The serum protease has been estimated by the amount of non-coagulable nitrogen formed per cc. of serum when emulsified with chloroform (8); the lipase by the usual ethyl butyrate method; and the antiferment titer by determining the amount of casein digestion by means of the Folin method, instead of the unreliable estimation by inspection of the precipitate. In the majority of the cases the type of organism has been determined by agglutination by means of specific sera.[2]

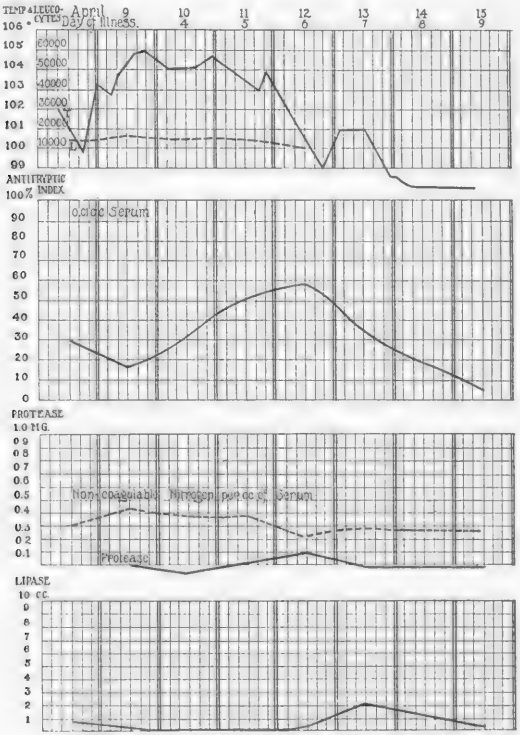
[1] Falls (Falls, F. H., *Jour. Infect. Dis.*, 1915, xvi, 466) has recently demonstrated such ferments in a series of cases. He concludes that the source of the ferments is not to be found in the leucocytes.

[2] These specific sera were kindly supplied by Dr. Cole of The Rockefeller Institute. We wish furthermore to express our appreciation to Drs. McCabe, Manier, and Weaver, and the members of their respective staffs of the City, Vanderbilt, and Industrial School Hospitals, who have placed their patients at our disposal for study and have facilitated our work in every way.

Case Records.

The following case records and their accompanying text-figures are typical of the relations.

Case 1 (*19*).—(Text-fig. 1.) J. H., male, colored, aged 28. Became sick Apr. 6, 1915, when he had a chill lasting a half hour, together with severe pain in both sides of chest. Entered hospital Apr. 7, 1915.



TEXT-FIG. 1. Serum changes during pneumonia (Case 1).

Physical Examination.—Well nourished colored man. Mucous membrane of mouth red, tongue coated, pharynx engorged. Pulse rapid. Lungs: diminished expansion on the left side, distinct tubular breathing, also vocal fremitus and dullness on percussion. Apr. 8, 1915. Urine negative except for a few granular casts.

The case progressed normally. On the 6th day the temperature began to decline and after a slight increase the following day reached normal the same evening.

The following facts will be noted from Text-fig. 1. The antiferment curve reached its maximum on the sixth day of illness, following which it showed a progressive decline. The protease reached a maximum on the sixth day; *i. e.*, when the temperature began to decline. At this time also the non-coagulable nitrogen showed a considerable drop. Following the fall in temperature the protease remained at a low level. The serum lipase declined progressively until immediately after the crisis when it increased almost to the strength of normal serum, with a following decline. The proteoses contained in the serum gave the following results (per 5 cc. of serum):

Date.	*mg.*
Apr. 8, 1915	0.25
" 10, "	0.21
" 13, "	0.13

The organisms isolated from the sputum agglutinated with serum of Type I.

Case 2 (16).—(Text-fig. 2.) J. W., male, colored, aged 18. Present illness began about 2 p. m., Mar. 16, 1915, with a chill and headache, aching of limbs and body, pain in left side and chest below the heart, accompanied with shortness of breath and expectoration of a dark brown sputum, and sometimes fresh blood. Admitted to the hospital Mar. 20, 1915.

Physical Examination.—Strong, well developed young negro. Head examination negative. Heart rapid; pulse rather weak and running in character. Blood pressure, systolic 98. Lungs: increased voice and breath sounds in both lower and middle lobes of right lung; grunting at the end of expiration; some bubbling râles over right lung anteriorly. Abdomen soft and slightly tender. Liver and spleen negative.

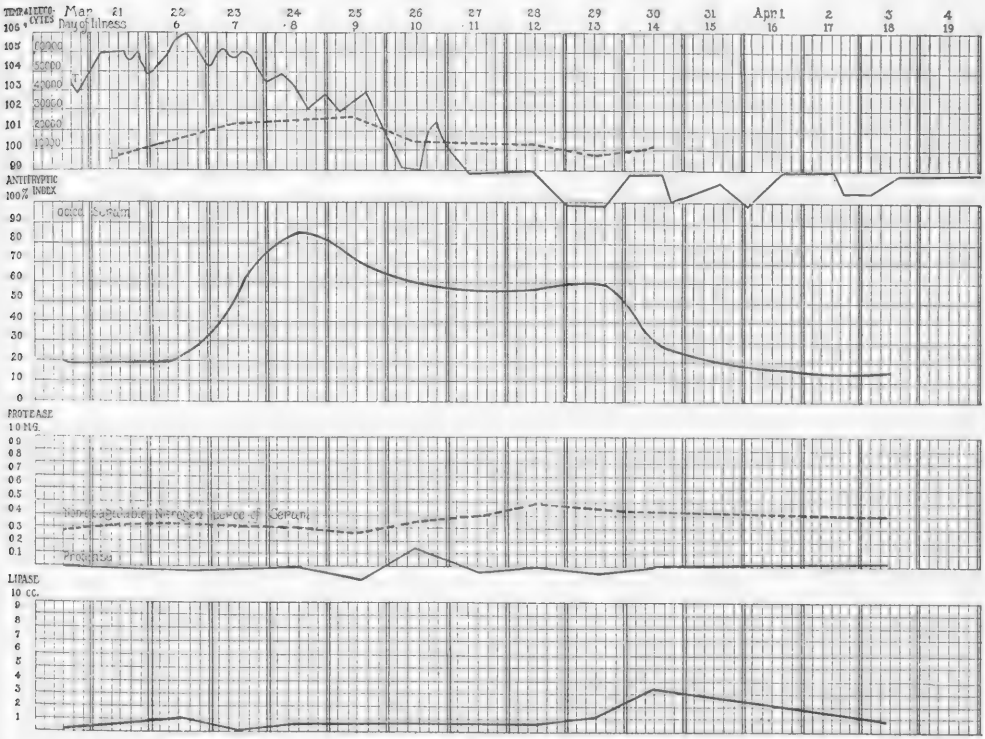
Pneumococcus isolated from the sputum agglutinated with serum of Type I.

On Mar. 23 the patient became delirious and remained irrational for the following 6 days, during which time it was necessary to restrain him in bed.

Text-fig. 2 shows that the greatest rise in antiferment occurred after the 6th day of illness and remained high until the 13th day. The non-coagulable nitrogen reached the maximum on the 12th day; after that it declined slowly. The protease change is quite striking. There was a lack of protease action during the entire time before the crisis. On the day before the crisis the total non-coagulable nitrogen of the serum incubated under chloroform

decreased from 0.27 to 0.17 mg. per cc. of serum. A control made by means of the Van Slyke method gave the following result, thus checking very well with the Folin determination:

	mg.
Amino nitrogen, 2 cc., direct	0.872
" " after incubating, 2 cc., direct	0.692
Loss	0.180 mg. per 2 cc.


Mar. 21 22 23 24 25 26 27 28 29 30 31 Apr. 1 2 3 4
Day of Illness
6 7 8 9 10 11 12 13 14 15 16 17 18 19
TEMPERATURE
LEUCOCYTES
ANTITRYPTIC INDEX
PROTEASE
LIPASE
10 cc.

TEXT-FIG. 2. Serum changes during pneumonia (Case 2).

On the following day, however, we noted a marked protease action, after which the ferment was again practically negative. The lipase reached the lowest titer on the 7th day, after which there was a gradual increase until the 14th day.

Case 3 (*12*).[3]—O. B., male, white, aged 18. Mar. 23, 1915, patient had a chill followed by a headache; pain in chest, especially on the right side; coughed a good deal, expectorated colored sputum. Admitted to the hospital Mar. 25, 1915.

Physical Examination.—Strong, robust boy. Head, heart, arteries, and pulse negative. Lungs: increased fremitus and voice sounds, marked increase over the right lobe, breath sounds also increased, percussion very flat. Abdomen slightly tender. Liver and spleen not palpable. Blood pressure, systolic 128. Urine contained albumin and granular casts. Clinical course was uneventful. The temperature fell by lysis, reaching normal on the 8th day.

The antiferment titer, which was relatively low, reached a maximum on the 6th day after which it declined progressively. The maximum of non-coagulable nitrogen was reached on the 6th day, declining slowly after that time. The protease was strongly positive on the 6th and 8th days of illness, corresponding to the period of lysis. The lipase reached the maximum on the 6th day, after which it increased almost to normal.

Case 4 (*17*).—G. T., male, white, aged 18. Patient was well and at work when without seeming cause he had a hard chill about 3 p. m. on Apr. 3, 1915. The chill continued through the night and until about 9 o'clock the next morning, together with pain in axillary region. After the chill he states that he perspired; had dyspnea; developed a cough at the time of the chill which became worse after the chill. Admitted Apr. 5, 1915.

Physical Examination.—White boy, fairly well nourished. Head negative; throat somewhat engorged. Heart normal; P. M. I. in the fifth interspace, nipple line; pulmonic second accentuated; arteries in good condition. Inspection shows decreased expansion on the left side and lower lobe both in front and behind; increased tactile fremitus; vocal resonance and tubular breathing on auscultation. Vocal fremitus is at first absent over the lower lobe on the left side, but is noted on change of position and after coughing. Abdomen normal. Urine negative. The crisis occurred on the 7th day.

In this case the antiferment did not reach a maximum until the day following the crisis, after which it declined. The non-coagulable nitrogen was never very high and showed little change. The protease before the crisis was negative; at the time of the crisis it became normal. The lipase showed the usual change. Proteoses per 5 cc. of serum:

Date.	*mg.*
Apr. 6, 1915	0.14
" 9, "	0.0

[3] This case was from the City Hospital.

Case 5 (*20*).[4]—R. M., male, colored, aged 20. Present illness commenced the last week in March with pain in the right side. He had no chill but vomited the next day. Since he became ill he has been in bed at a railroad camp; is somewhat constipated; has coughed a good deal, expectorating much sputum, sometimes blood tinged; has pain, now worse than at the onset on each inspiration and when he coughs, more especially over abdomen and lower chest on right side; never had a chill.

Physical Examination.—Negro, poorly nourished. Head negative. P. M. I. inside of nipple line in fifth interspace; sounds are regular, no murmurs; second pulmonic accentuated; pulse regular and full, and of good volume. Blood pressure, systolic 103, diastolic 60. Lungs: respiration rapid, decreased expiration on the right side; tactile fremitus increased over right lower lobe together with decreased voice sounds, dullness, and tubular breathing. There is a friction sound behind and over the right lower lobe. The middle lobe of the right lung is quite flat with some tubular breathing, increased voice sounds, and fremitus. The left lung is normal. The abdomen is rigid and tender and the lymph glands especially are enlarged. Apr. 9, 1915. Urine examination: albumin negative; numerous casts. Apr. 14, 1915. Some hyaline and granular casts. Apr. 8, 1915. Blood examination: red cells 4,800,000; hemoglobin 70 per cent. Apr. 9, 1915. Condition worse; temperature has risen to 103° F., and pulse to 140. Examination shows no change over right middle lobe; right lower lobe still shows impairment with bronchovesicular breathing and increased voice sounds, except the lower part in posterior axillary line, where there is flatness with diminished breath and voice sounds. The upper right lobe shows slight impairment with a tendency to bronchovesicular breathing; posterior shows nothing as yet definite. Left lung clear throughout. Heart sounds rapid and of only fair quality, though the second pulmonic is still accentuated. Needle was inserted next to right inner area but no fluid obtained. Blood pressure, systolic 116. Apr. 10, 1915. Patient much improved, temperature having fallen this morning to 99.4° F. and pulse to 110. Patient expectorated yesterday considerable brownish material. Examination to-day shows much less impairment over middle lobe except in region of anterior axillary line where it is still flat with tubular breathing and exaggerated voice sounds; many moist râles over whole lobe. Lower lobe is clearing up except at base where there are still diminished breath sounds and voice sounds. Blood pressure, systolic 105, diastolic 60. Apr. 11, 1915. General condition excellent. Temperature is normal and pulse 100. Examination shows but little impairment over middle lobe, with only slight voice sounds and breath changes. Apr. 12, 1915. Blood pressure, systolic 95.

As will be noted, the patient entered the hospital practically at the end of his illness, the antiferment and non-coagulable nitrogen declining very rapidly, while the protease tended to rise. 5 cc. of serum contained 0.2 mg. of proteoses on April 11, 1915.

[4] This case was from the Vanderbilt Hospital.

Case 6 (21).[5]—H. H., male, white, aged 18. In 1913 patient had rheumatism in both arms and legs; was ill in bed about a month. Present illness began Apr. 6, 1915, with pain on left side in nipple region. Apr. 7, 1915. Had chill and high fever, expectorated, cough was dry and hacking. Since then cough has been deep seated and he has expectorated more than at first. Entered hospital Apr. 9, 1915.

Physical Examination.—Fairly well nourished. Head negative. P. M. I. normal, arteries soft and compressible, accentuated pulmonic second sound. Lungs: deficient expansion on left side, decreased fremitus over left upper lobe in front, dullness and feeble breath sounds over left upper lobe, tubular breathing in the left axilla. Blood pressure, systolic 95. Crisis on the 8th day. General condition excellent throughout.

The antiferment titer reached a maximum on the 6th day, after which it declined until the day following the crisis. The non-coagulable nitrogen reached its maximum on the 5th day, at which time the protease was lowest. The protease reached + 0.02 mg. per 1 cc. on the day following the crisis. The lipase showed the usual curve. Proteoses per 5 cc.:

Date.	*mg.*
Apr. 9, 1915	0.19
" 13, "	0.12
" 14, "	0.13

Pneumococcus of Type I was isolated from the sputum.

Case 7 (18).—T. A., male, colored, aged 30. Present illness began with headache about a week ago. On Apr. 5, 1915, 3 or 4 days following headache, he had chill about 10 a. m., which lasted for about 3 hours; no vomiting, but fever following chill; pain in the right side. Admitted Apr. 6, 1915.

Physical Examination.—Well nourished colored man. Head negative; some pyorrhea; pulse 120, but full in volume. P. M. I. fifth interspace mammary line; no murmurs. Increased fremitus and voice sounds on the right side in front, no dullness on percussion. Abdomen rigid, otherwise negative.

The patient showed a rather irregular course. On the 7th day the temperature dropped to normal, but he complained of a severe sore throat the same evening and a marked tonsilitis was noticed. This continued for 3 days. After this time the temperature again reached normal. Apr. 17. Pulse 118, respiration 30. Tonsils engorged, with some exudate. Lungs: fremitus increased over upper and middle right lobes; very dull; occasional moist râle. Breath sounds bronchial. This case is rather interesting because of the period on the 7th day when it appeared clinically that the patient was having a crisis.

At this time the non-coagulable nitrogen had decreased and the protease reached + 0.05 mg. per 1 cc. The antiferment also

[5] This case was from the City Hospital.

showed a slight fall from the height reached on the previous day. Then, however, the tonsilitis set in and we found the antiferment continuing at a high titer, while the non-coagulable nitrogen increased again and the protease fell. The resolution seems to have been delayed, for the clinical signs began to clear only after April 17, when the protease again reached + 0.05 mg. per 1 cc. of serum. The proteoses determined per 5 cc. of serum gave the following figures:

Date.	*mg.*
Apr. 6, 1915	0.125
" 14, "	0.26
" 16, "	0.13
" 18, "	0.00

Case 8 (14).[6]—W. A., male, colored, aged 35. Present illness began Mar. 31, 1915, with pain in the left side, coughing, and expectoration of a bloody sputum; he lost his appetite but has not been nauseated; there was diarrhea; no chill. Admitted to hospital Apr. 1, 1915.

Physical Examination.—Well nourished young man. Teeth bad. Heart normal; second pulmonic accentuated. Lungs: tactile fremitus normal on both sides, except in front on left side where it is increased downward and outward from the left nipple line. Percussion is normal except for the above mentioned area, which is very dull. On auscultation, vocal fremitus is normal except over the same area where it is increased; there is bronchophony and tubular breathing on inspiration and expiration, together with coarse râles. Abdomen is negative. Blood pressure, systolic 90 and diastolic 50. Urine shows few hyaline and granular casts. Apr. 1, 1915. Red blood cells 5,000,300. Apr. 4, 1915. Condition good. Temperature dropped suddenly to normal at 9 a. m., pulse and respiration dropping with it. Signs over original area practically cleared up, beyond moist râles. No impairment and no tubular breathing; no signs over rest of lower lobe, beyond a few râles. Over left upper lobe there is flatness, with exaggerated tactile and voice frictions and bronchial breathing; moist râles are heard over this area. Blood pressure, systolic 80, diastolic 50. Apr. 5, 1915. Temperature went up again last evening to 101° F. Examination this morning shows complete consolidation of the upper lobe with exaggerated tactile fremitus, vocal resonance, and bronchial breathing; original area shows no change in signs. Abdomen quite distended. Apr. 6, 1915. Temperature down around 99° F., pulse slow and of better volume. Patient is resting better. Pain in side not severe; signs still present on left side. Blood pressure, systolic 95, diastolic 60. Apr. 7, 1915. Blood pressure, systolic 80. Apr. 8, 1915. General condition good. Temperature has been practically normal for last 2 days. Signs over lower left lobe have practically cleared up; there is some slight impairment over left upper lobe with bronchovesicular breathing and a few râles. Apr. 11, 1915. Condition good. Temperature has been normal

[6] This case was from the Vanderbilt Hospital.

now for almost 4 days. Examination of lung to-day shows no impairment or evidence of previous trouble except occasional râles at end of inspiration.

The antiferment curve reached its maximum at the time the temperature first fell to normal. It is quite evident, however, that at this time the patient had no crisis, for the leucocytes continued to rise until the following day. The non-coagulable nitrogen, however, was much lower after the fifth day of illness. The protease was positive on the sixth and ninth days of illness; after that time the patient made an uneventful recovery. The lipase was rather high throughout.

Case 9 (29).[7]—H. G., male, colored, aged 35. Illness began Apr. 6, 1915, when he began to cough a great deal, which caused pain in left side; expectorated much thick brownish sputum; had no chill. Entered the hospital Apr. 11, 1915.

Physical Examination.—Icteric; mucous membrane of mouth red; tongue coated; heart and arteries normal. Lungs: left lung shows deficient expansion, tactile fremitus increased, and large râles and spoken voice sounds heard in both upper and lower lobes, together with dullness on percussion. Abdomen negative. Reflexes normal.

This patient is the only one in the series that we have studied who showed a constant amount of protease in the serum during the disease and showed no change during the crisis, unless it was a slight fall. The antiferment remained high even after the crisis. The lipase remained low throughout. Proteoses per 5 cc. of serum:

Date.	*mg*
Apr. 16, 1915	0.21
" 17, "	0.12

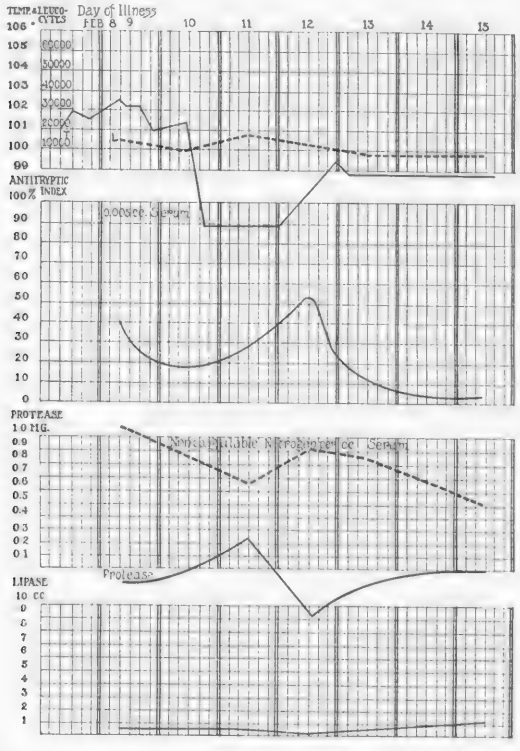
Case 10.[8]—(Text-fig. 3.) J. M. J., male, white, aged 40. Became ill Jan. 31, 1915, when he had a chill. Entered hospital Feb. 5, 1915.

Physical Examination.—Typical right lower lobe involvement. Urine contained numerous casts. White blood count 10,200. Sputum contained numerous pneumococci (type not determined). The patient showed evidence of profound intoxication, was stuporous, with rapid pulse and respiration. On the 10th day the temperature dropped by crisis, but the physical condition, especially the pulse rate, continued unfavorable. From the 9th to the 12th day the patient was delirious. After the 12th day the recovery was rapid.

[7] This case was from the City Hospital.

[8] This case was from the City Hospital.

The course of the disease was marked by a rather low leucocytosis and a large amount of non-coagulable nitrogen in the serum. The temperature fell as the amount of protease increased, but there was considerable intoxication for the three days following, as evidenced



TEXT-FIG. 3. Serum changes during pneumonia (Case 10).

by the increase in antiferment, the drop in protease, and the rise in non-coagulable nitrogen. The lipase began to return toward normal after this second period of intoxication.

Case 11 (22).[9]—E. H., male, colored, aged 30. Patient came to hospital because of pain in lower right chest, which increased on deep inspiration; this was first noted Apr. 4, 1915, when coughing accompanied pain. Apr. 7, 1915.

[9] This case was from the City Hospital.

Had a chill and headache since that time. Admitted to the hospital, Apr. 9, 1915.

Physical Examination.—Head negative. Heart: apex slightly down and out from the normal point; no murmurs; second pulmonic increased; pulse full, rather hard but regular. Lungs: right, slight dullness of lower lobe in front; no increase in voice sounds and tactile fremitus; left lung normal. Blood pressure, systolic 110, diastolic 65.

There was a progressive rise in the serum protease, reaching a maximum on the seventh day of illness, at which time the non-coagulable nitrogen had largely decreased. The antiferment decreased after the seventh day. Proteoses per 5 cc. of serum:

Date.	*mg.*
Apr. 12, 1915	0.13
" 13, "	0.30
" 14, "	0.29
" 15, "	0.15

Pneumococci from sputum agglutinated with serum of Type I.

Case 12 (23).[10]—G. F., male, colored, aged 20. Apr. 4, 1915. Pain in back and left side on breathing; no chill; has had some fever and expectorates blood tinged sputum; some coughing; shortness of breath. Entered hospital Apr. 11, 1915.

Physical Examination.—Eyes and head negative. Arteries soft, heart normal. P. M. I. fifth interspace inside of nipple space. Lungs: dullness, tubular breathing, increased voice sounds, tactile fremitus over left lower lobe, friction sound heard over left lower chest. Abdomen rigid. Liver and spleen not palpable, otherwise normal.

The patient became irrational and delirious the second day after admission to the hospital. On the 11th day of illness the pneumonic process involved the upper lobe on the same side, evidenced by area of tubular breathing and dullness. The patient died on the 15th day. No autopsy was obtained. A pneumococcus agglutinating with serum of Type II was obtained from the sputum.

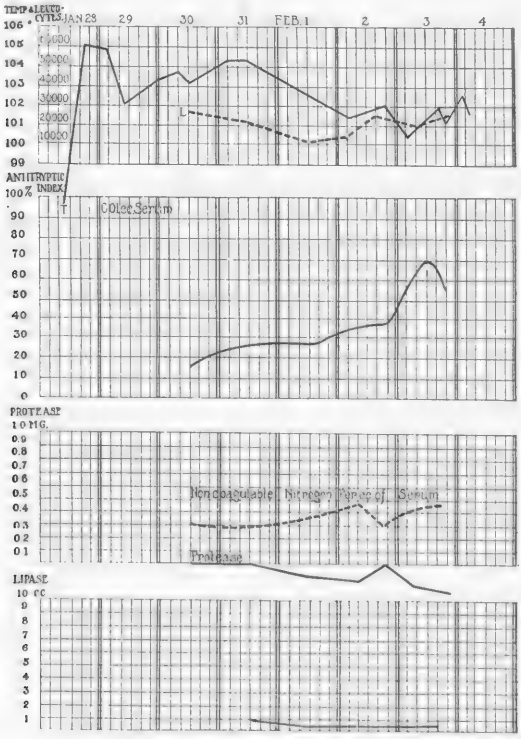
There was a rather constant increase in antiferment, which reached a maximum on the eleventh day and remained high after that time. The protease was low during the course of the disease but showed a gradual rise. The non-coagulable nitrogen remained relatively low throughout. Proteoses per 5 cc. of serum:

Date.	*mg.*
Apr. 12, 1915	0.1
" 16, "	0.0

[10] This case was from the City Hospital.

Case 13.[11]—(Text-fig. 4.) J. H. D., male, colored, aged 14. Jan. 28, 1915, 3 a. m. Became suddenly ill with a severe chill and pain on the right side of chest. Has coughed for the past few days. Admitted to hospital 9 a. m., Jan. 28, 1915.

Physical Examination.—Well nourished negro boy. Physical findings negative. No evidence of consolidation. White blood count 40,000. Jan. 29. Dullness and bronchial breathing were noted over entire right lung. The patient became very restless and coughed considerably. Jan. 30. All evidences of a complete consolidation of the entire right lung were present. There was con-



TEXT-FIG. 4. Serum changes during pneumonia with fatal termination (Case 13).

siderable pain and restlessness. Feb. 3. In the morning the patient showed definite signs of improvement, was quiet, and numerous large râles were noted over the involved area. During the following night, however, he became much worse and died at 5.30 a. m.

[11] This case was from the Vanderbilt Hospital.

Data from the Postmortem Protocol.—Body is that of a negro boy 14 years old, 172 cm. in length, well developed, and well nourished.

Lungs.—The right lung weighs 1,080 gm. The surface is smooth with the exception of the posterior part of upper lobe. There are many soft, friable adhesions. There is a small amount of amber-colored fluid in the pleural cavity. The lung when removed from the chest maintains its form. There are definite areas of consolidation in the lower part of the upper lobe. The middle lobe is free. On section through these consolidated areas the surface yields very little fluid and the lung tissue is definitely consolidated, of a grayish appearance, and around the margins of each there is a red zone. The left lung weighs 740 gm. The pleura is smooth. The upper lobe is normal. The lower lobe shows areas of consolidation similar to those described in the right.

Anatomical Diagnosis.—1. Lobar pneumonia of both lower lobes and bronchopneumonia of the right upper lobe. 2. Fibrinous pleuritis. 3. Acute splenitis. 4. Fatty changes and cloudy swelling of the liver, kidneys, and myocardium.

It will be seen from Text-fig. 4 that there was a progressive increase in the non-coagulable nitrogen until the evening of February 2, when a fall occurred. At this time there was also a distinct change in the ferment, so that on incubation a loss of non-coagulable nitrogen no longer occurred, although the serum did not actually become proteolytic. Instead, the non-coagulable nitrogen again increased and the ferment fell while the antiferment increased markedly. It will be observed that clinically the patient was in good condition during the night and morning after the temporary fall in non-coagulable nitrogen and the rise in ferment. The lipase showed simply a continued decrease.

Case 14.[12]—H. M., male, colored, aged 30. Became ill with chill and thoracic pain on Dec. 11, 1914. Entered hospital Dec. 15, 1914.

Physical Examination.—Breath sounds diminished on left side, with vocal fremitus increased over right upper lobe posteriorly. Pulse rate 108 to 120. Heart normal. Patient continued to run a typical course and the temperature dropped to normal on the 9th day, the respiration dropping from 38 to 24, and the pulse rate from 132 to 108 from the afternoon of the 19th to the morning of the 20th day. The temperature, however, again rose and continued to be irregular. Dec. 28, 1914. Patient developed meningeal symptoms. Lumbar puncture at this time yielded a small amount of turbid fluid. The pressure was not increased, nor were organisms obtained on examination. Dec. 31, 1914. Patient died.

Autopsy.—Dec. 31, 1914.

Heart.—Weight 450 gm. The tricuspid valve measures 14 cm. The pulmonary valve measures 7 cm. The cusps of the mitral valve contain vegetations measuring from 2 cm. in diameter to others which are smaller. These vegeta-

[12] This case was from the City Hospital.

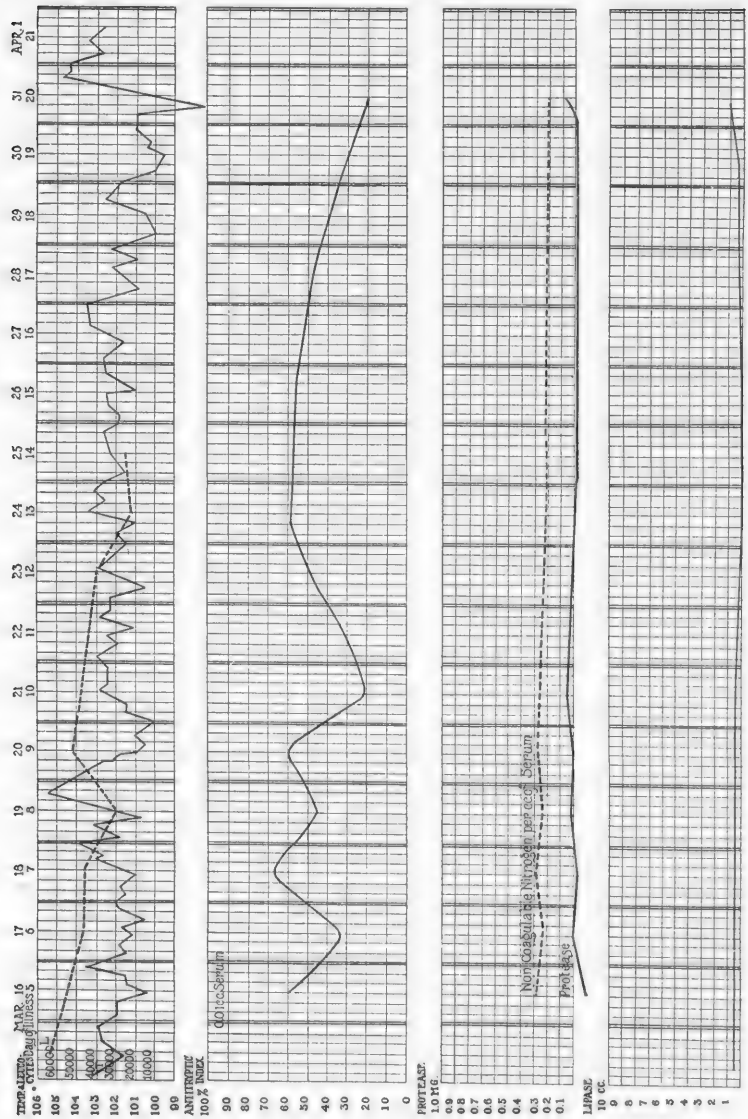
tions are firmly attached to the cusps. The distal surface is roughened and corrugated; otherwise they present a smooth surface. The cusps of the aortic valve are smooth and shining. The myocardium is brownish red in color and presents several grayish white patches, which are evidently fibrous in character. The left ventricle measures 17 mm., the right ventricle 7 mm. in thickness.

Lungs.—The left lung weighs 840 gm. The lung is entirely covered with a fibrous and fibrinous exudate, which binds it to the chest wall. It is bluish black in color. The consistency is increased and the organ contains no air except in the apex. The bronchi are bright red in color and contain a mucopurulent exudate. On section the surface is dark gray in color. In the lower lobe the process is diffuse. In the upper lobe it is nodular in character. The centers of the nodules present a grayish color and the peripheries are red. The cut surface is rather smooth. The right lung weighs 500 gm., and is bluish black in color. The edges are rounded. The surface is smooth. The upper and lower lobes are bound together by old fibrous adhesions. The lung is markedly emphysematous. On section the surface is pinkish gray in color and dry.

Brain.—On removing the dura the cortex is seen to be covered with a greenish, cloudy, fibrinous exudate. This involves the frontal and parietal lobes of the cortex. Occiput shows more congestion than the other two named. The fluid is increased along the sulcus and especially along the Rolandic fissure. In the fluid are flakes of a purulent material. The intima is firmly adherent to the convolutions of the brain and the exudate extends between the convolutions. The base of the brain shows the same condition as the cortex but apparently more marked in the infundibulum and the medulla oblongata. The lateral ventricle contains a thick, cloudy fluid. The choroid plexus is covered with an inflammatory exudate. The third ventricle is filled with the above described fluid. Upon section the blood vessels in the cerebellum are engorged and in many places there are many small petechial hemorrhages. The blood vessels medially to the external capsule are very much engorged. The purulent exudate is also found in the fourth ventricle.

Anatomical Diagnosis.—1. Lobar pneumonia of the left upper and lower lobes. 2. Pneumococcus meningitis. 3. Vegetative endocarditis. 4. Acute parenchymatous nephritis. 5. Cloudy swelling of the myocardium, liver, and spleen. 6. Fatty changes of the liver. 7. Fibrous and fibrinous pleuritis. 8. Emphysema of the right lung. 9. Fibrous myocarditis.

Microscopical Diagnosis.—Heart shows an increase in fibrous tissue, the muscle fibers are pale, enlarged, and some of them, especially beneath the pericardium, show fatty degeneration. One lung showed congestion, anthracosis, and compensatory emphysema; in the other lung the pleura was thickened, and many of the alveoli of the lung were collapsed, some of them showing fibroblasts and fibrous tissue. There was intense congestion and the bronchi were filled with an acute inflammatory exudate. Liver showed congestion, a few areas of necrosis, and some cloudy swelling. The capsule of the spleen is thickened; there is much congestion; and an increase in parenchymatous tissue. The capsule of the kidney is thick, glomeruli are swollen, the epithelium of the tubules is swollen, granular, and contains fat. Pancreas is negative. Stomach shows no definite change. The cerebrum is covered with an inflammatory exudate, rich in polymorphonuclear leucocytes. The pia mater is markedly thickened,



TEXT-FIG. 5. Serum changes during secondary pneumonia with fatal termination (Case 15).

the cortex is edematous, and there is marked perivascular infiltration of polymorphonuclear leucocytes.

This case showed the usual progressive rise in antiferment to the seventh day of illness, and the temperature reached normal on the ninth day. The protease was only determined three times, showing a progressive rise, however, about the time of improvement of the condition clinically. No signs of resolution were noted, however; the antiferment again rose, as did also the non-coagulable nitrogen. The lipase, except for an early rise, remained low. The high antiferment index may possibly be associated with the lack of autolysis in the lung described above.

Case 15.[13]—(Text-fig. 5.) J. T., female, colored, aged 14. Mar. 6, 1915. Abortion of 3 months' fetus, followed by fever 3 days later, together with dyspnea, cough, and general malaise. Entered hospital Mar. 12, 1915.

Physical Examination.—Young mulatto girl, well nourished. Heart action vigorous and rapid; second pulmonic sound accentuated; slight systolic thrill; no murmurs. Lungs: diminished expansion on left side; increased tactile fremitus over left upper lobe; dullness over lower portion of left upper lobe posteriorly and in axilla; increased voice sounds, bronchial breathing; and a few râles over lower portion of upper left lobe. Mar. 16. The entire left lobe became involved, with more numerous râles over the upper lobe. Mar. 17. There was distinct evidence of involvement of the lower right lobe, and the pulmonic second sound was found less accentuated. Mar. 18. Some signs of resolution were noted in the left lower lobe. Mar. 19. Hemorrhage. Mar. 20. Heart sounds very weak; patient in poor condition. The following days showed considerable resolution, but the cough continued, and the sputum became rather offensive. Apr. 1. Patient in a very critical condition. Patches of bronchial breathing and bronchophony over posterior part of both upper lobes, and in front on left side, with a patch in the right axilla. Great amount of offensive sputum. Died in the evening after a rather profuse hemorrhage.

In this case of septic pneumonia the fluctuating involvement of the lungs finds its reflection in the constantly changing antiferment curve, and the non-coagulable nitrogen also shows an almost daily variation. The protease remained low throughout, despite the clinical evidence of resolution in part of the involved areas. An autopsy was not obtained.

Analysis of Cases.

On examination of Text-figs. 1 to 5 there will be noted a very constant increase in the serum protease about the time of crisis, together

[13] This case was from the Vanderbilt Hospital.

with a decline in the antiferment titer. These two factors would indicate that at this time conditions for autolysis would be most favorable, and we must remember that the sera are after all only an index of the condition that obtains in the local areas involved.

It will be noted that the non-coagulable nitrogen decreases, as a rule, after the crisis, as Rzetkowski (9) has already pointed out, and the proteoses also diminish markedly.

When we average the various changes that have occurred in the eight cases that ended by crisis we obtain the figures given in Table I.

TABLE I.

Average Antiferment Titer.

Day before crisis.	Day of crisis.	Day following crisis
per cent	*per cent*	*per cent*
57	50	46

Average Total Non-Coagulable Nitrogen per Cc.

mg.	*mg.*	*mg.*
0.50	38	40

Average Protease Action per Cc. (Autolysis under Chloroform).

mg.	*mg.*	*mg.*
— 0.05	+ 0.05	+ 0.02

Average Lipase per Cc.

0.45 cc. $\frac{N}{100}$ NaOH	0.7 cc. $\frac{N}{100}$ NaOH	0.9 cc. $\frac{N}{100}$ NaOH

The protease activity which is demonstrated is probably not derived from the leucocytes undergoing autolysis in the pneumonic lung. If that were true we would expect that the ferment would continue to be present after the autolysis had once begun. That this is not the case is best illustrated in Text-fig. 5, when clinically all signs of resolution were present while the protease curve remained negative. The ferments are probably mobilized from the tissue cells in general.

DISCUSSION.

In a paper discussing the crisis in pneumonia, Hektoen (10) has recently reviewed the present status of our knowledge and concludes that the crisis is the effect of the prompt destruction of the infecting organism at a time when the antipneumococcal reaction reaches a certain height. But the mere concentration

of antibodies can hardly explain the whole picture, for in pneumonia, as in typhoid, such immune reactions may be quite marked, but the organism nevertheless may remain viable. He remarks that the crises cannot be wholly dependent on resolution, for, while they usually concur, the crises may precede resolution. But autolysis may take place in a lung without affording immediate evidence by physical finding, just as readily as the early infection may be well established in the lung before signs of consolidation can be elicited.

Dick (11) and Rosenow (12) have studied specific proteases both in the immune animal and in clinical material, Dick determining an increased proteolytic power for pneumococcus protein at the time of the crisis, and associating it with an increase in the complement strength. Even if such proteolysis were specific, rather than based on colloidal changes following in the wake of the usual immunity reactions, it would have no influence on living pneumococci, for proteolytic ferments, as is well known, are without action on intact organisms (13).

That autolysis plays a large share in the pneumonic picture has been shown by Müller (14). Flexner (15) has discussed more especially the conditions present in unresolved pneumonia.

Before entering into a discussion of the relation of autolysis to the crisis, we believe that our ideas concerning the factors that produce the intoxication must be amplified. Quite naturally the entire emphasis has so far been placed on the pneumococcus. Kruse (16) and Cole (17) have discussed the work so far accomplished in the study of pneumococcus toxicity. Vaughan (18) has shown that pneumococcus proteins are relatively less toxic than those of other organisms. The toxicity is manifested on simple solution, to which the pneumococcus is rather liable, *e. g.*, with bile salts (Neufeld), and soaps (Lamar); by freezing (Cole); lipoid solvents (Jobling and Strouse); and also during autolysis, as Rosenow has demonstrated (19). Rosenow determined the rate of autolysis by means of the Sörensen method.

Using the Folin method and coagulating by means of heat and acid, we have not been able to convince ourselves that the pneumococcus undergoes autolysis more readily than other organisms. In one experiment the following rate of autolysis to non-coagulable forms of nitrogen was noted:

	hrs.	*per cent.*
Pneumococcus	24	6
"	48	11
Typhoid	24	4
"	48	25
Staphylococci	24	30
"	48	45

It is possible that other strains might show some difference. The organisms used contained from 3.6 to 4.7 per cent of total lipoids; the degree of unsaturation was not determined because the amount of lipoids obtained was too small. If the pneumococcus at the time

of crisis was subject to extensive lysis and was the sole factor in the causation of the toxemia, we should expect an increase rather than a lessening in intoxication, for during lysis toxic material is liberated from the organism.

The fact which we wish to emphasize is the great mass of fibrin and leucocytic debris which dominates the pneumonic picture. This to all intents and purposes represents foreign proteins as far as the lung is concerned and while undergoing solution must give rise to toxic split products. While the inhibitory factors are in the ascendency, this material is undergoing a very slow autolysis with the splitting proceeding only to the higher and toxic products which are absorbed as such. The balance being once destroyed, and the autolytic process allowed full sway, the splitting proceeds much farther to non-toxic products. This period is characterized clinically by the greatest excretion of nitrogen following immediately upon the crisis. We believe that it is this factor (to which Cole (20) has already referred) that must be taken into consideration when we seek to define the various potentially pyrogenic sources.

We regard the amount of proteoses present in the serum as an indication that such a condition actually obtains. It will be noted from the various determinations made that these reach two or three times the amount present in the early serum samples and fall off quite markedly after crisis. These must be derived either from the resolving lung tissue, or the tissue cells in general, for their amount is too large for the pneumococcus protein to be considered their source. It is recognized clinically that fibrin and blood clots or aseptic tissue autolysis in general give rise to a febrile condition as readily as an accumulation of leucocytes among which the infecting organism may have completely disappeared. The source of the toxin here is quite palpably the autolyzing homologous proteins. It seems only reasonable, therefore, that the same condition, even in an exaggerated form, should, during the pneumonic process, give rise to an intoxication. Under such circumstances the pneumococcus intoxication proper would be only an accessory factor, and would account for the fact that the rapid destruction of the organisms which must occur during and after the crisis is accompanied by no further evidence of intoxication.

CONCLUSIONS.

1. The crisis in pneumonia is usually accompanied by (a) decrease in the serum antiferment; (b) the mobilization of a non-specific protease in the serum; (c) an increase in serum lipase; (d) a decrease in the non-coagulable nitrogen, and of the proteoses in the serum.

2. The crisis is associated with the beginning of an active autolysis, the latter depending on an altered relation between the ferment-antiferment balance.

3. The fibrin and leucocytic debris must be considered as one of the potential sources of toxic substances. With rapid autolysis proceeding, only non-toxic materials are absorbed.

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SERUM CHANGES FOLLOWING KAOLIN INJECTIONS.

Studies on Ferment Action. XXV.

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(Received for publication, May 19, 1915.)

The observation of Keysser and Wassermann (1), that the serum of certain animals becomes toxic when incubated with an inert substance such as kaolin, has stimulated considerable interest in the mechanism of the reactions that occur. Keysser and Wassermann considered the phenomenon due to an adsorption of amboceptor. Gengou had noted that quite indifferent substances (he used barium sulphate and calcium fluoride) were hemolytic and that this effect was held in abeyance by serum.

Friedberger and Kumagai (2) made similar observations with kaolin and determined that the serum albumin and globulin protected against the hemolytic effect, while the split products of the proteins protected in a degree corresponding to their complexity. They decided that the inhibiting substance of the serum was not lipoidal, for they found that if they washed the serum ten times with ether the serum still protected. They do not explain the loss of the inhibitory effect of the serum, for if this is due solely to protein bodies we can hardly see why single or even repeated treatments with the kaolin should exhaust the serum.

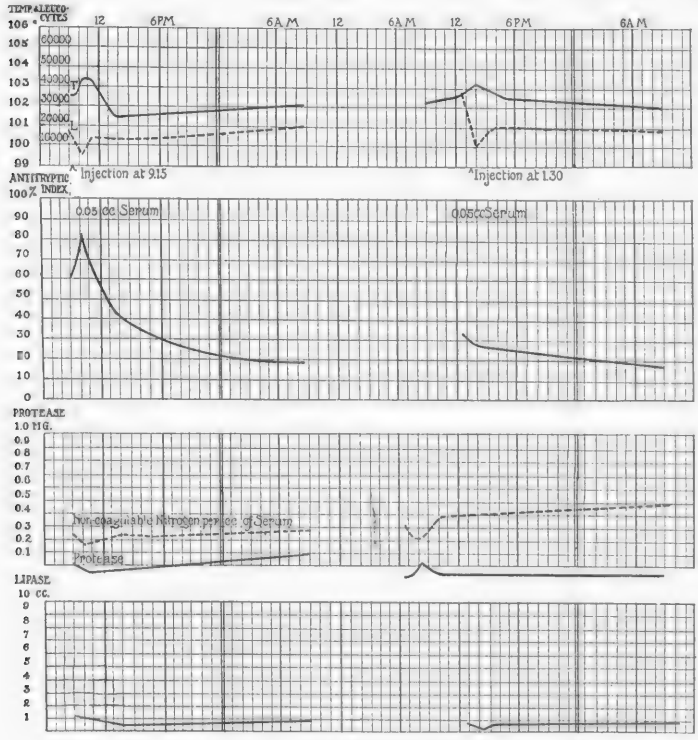
Friedberger at first considered the effects of intravenous injection of inert substances, such as kaolin, as purely mechanical, *i. e.*, plugging of the capillaries of the brain and lung, in this way leading to an acute death. Later, however, together with Tsuneoka (3) he admitted that the toxic effect could not be so interpreted, for after treatment with serum the kaolin was non-toxic.

In previous papers (4) we have developed the idea that it is the serum antiferment which is most readily adsorbed from the serum by inert substances such as kaolin and barium sulphate. By such adsorption local areas of antiferment deficiency are formed where proteolysis can take place, with a resulting production of toxic split products. Inasmuch as this is the mechanism in anaphylatoxin formation we might expect that the intravital injection might give evidences of a similar condition. The following experiments have been carried out with this point in view.

EXPERIMENTAL.

Dog 11.—Weight 5.5 kilos. 0.05 gm. of kaolin suspension was injected intravenously at 9.15 a. m. The animal showed evidence of malaise; was nauseated; temperature increased slightly after 1 hour but fell in the afternoon.

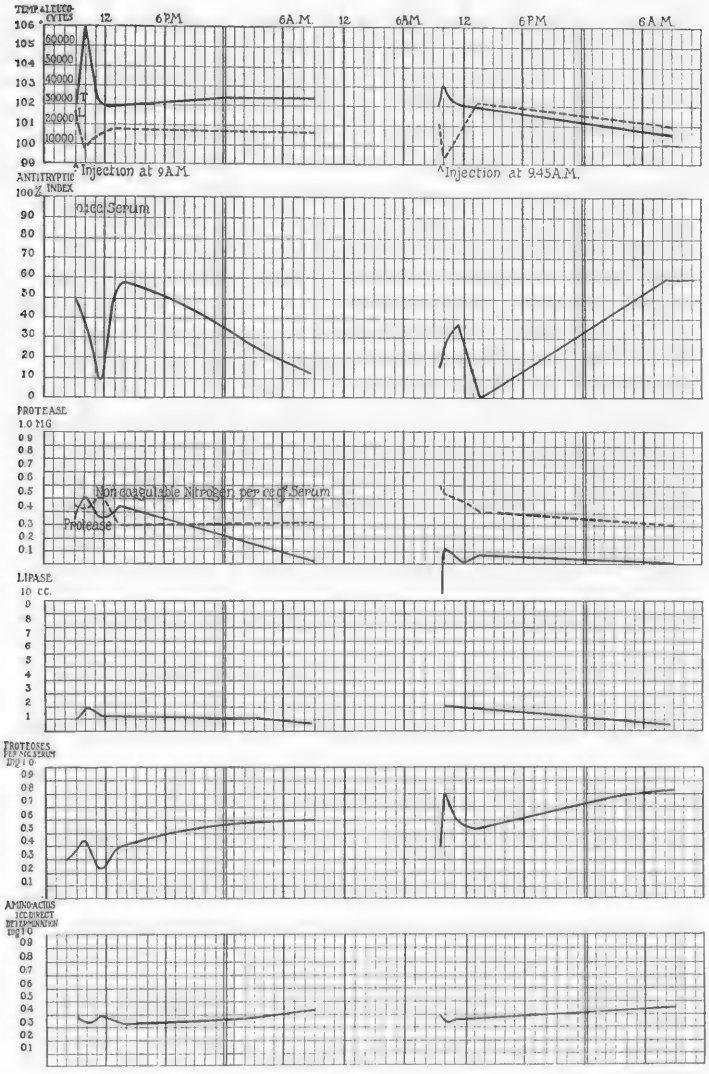
From Text-fig. 1 it will be observed that there was at first a sharp rise in the antiferment, followed by a gradual decline. The serum lipase showed a slight decrease, as did also the protease. A second



TEXT-FIG. 1. TEXT-FIG. 1 a.

TEXT-FIGS. 1 AND 1 a. Serum changes following intravenous kaolin injections.

injection was made 4 days later (Text-fig. 1 a), double the amount of the first injection being used (0.1 gm.). The temperature and the leucocytic reaction were similar, although the antiferment



TEXT-FIG. 2. TEXT-FIG. 2 a.

TEXT-FIGS. 2 AND 2 a. Serum changes following intravenous kaolin injections.

showed less alteration. In the afternoon there was a considerable increase in the non-coagulable nitrogen in the serum. The protease was only temporarily increased.

Dog 66.—Weight 6 kilos. 0.5 gm. of kaolin was injected at 9.45 a. m. (Text-fig. 2 a). This amount caused complete prostration with nausea, vomiting, and diarrhea. The blood taken 15 minutes after the injection remained fluid.

As will be noted from Text-fig. 2 a there was an immediate rise in antiferment, followed by a secondary fall with recovery the following morning. The non-coagulable nitrogen decreased while there was some increase in the protease, which remained low throughout. The lipase showed a gradual decrease. There was an immediate increase in the proteoses in the serum, while at the same time a slight decrease in amino-acids was noted.

Dog 63.—Weight 6.3 kilos. Sensitized Apr. 2, 1915, with horse serum albumin. 0.5 gm. of kaolin was injected at 9 a. m., Apr. 21, 1915.

This animal differed in its response in the greater rise in temperature and an immediate fall in antiferment (Text-fig. 2); indeed the antiferment curve is exactly the reverse of that of Dog 66. There was noted an immediate rise in proteoses, while the amino-acids decreased slightly as in Dog 66.

The following dogs (Table I) received doses which were almost immediately followed by evidence of a profound shock and an early death. In each case the symptoms were identical.

TABLE I.

Dog No.	Weight. *kilos*	Injection.	Result.
67	5.0	1 gm. kaolin in suspension	Died, 10 min.
65	6.2	0.8 " " " "	" 20 "
68	6.2	0.6 " " " "	" 5 "
69	6.0	0.4 " " " "	" 15 "

The blood examinations immediately before the injection and at the time of death are given in Table II.

It will be observed that there is a distinct increase in the amount of protease in the serum, as also of the higher split products, while the amino-acids show practically no change. The antiferment titer is markedly increased.

TABLE II.

Dog No.	Time.	Total non-coagulable nitrogen per cc.	Protease action per cc.	Proteoses per 5 cc. of serum.	Amino-acids per cc. (direct determination).	Lipase per cc.	Antiferment inhibition.
		mg.	*mg.*	*mg.*	*mg.*	*cc. N/100NaOH*	
67	Before injection	0.28	0.22	0.5	0.32	0.5	17% per 0.1 cc.
	After "	0.32	0.30	0.74	0.30	0.2	50% " " "
65	Before "	0.28	0.04	0.45	0.41	2.2	33% " 0.2 "
	After "	0.28	0.37	0.41	0.42	2.2	75% " " "
69	Before "	0.31	0.37	0.55			
	After "	0.32	0.68	0.83			

DISCUSSION.

The experiments which have been detailed above give evidence that the toxicity of an inert substance such as kaolin depends on adsorption phenomena which result in protein splitting. Friedberger and Tsuneoka consider that an adsorption of certain substances from cells essential to life takes place. If that were the case we should expect that the serum would protect against the toxic effect, or, if this protection were not sufficient, that the red cells would be the first to be injured with a resulting hemolysis. This, however, never takes place *in vivo,* even during the acute shock. On the other hand, the shock bears much resemblance to the intoxication brought about by protein split products in its effect on coagulation, gastro-intestinal intoxication, the immediate leucopenia, etc. If this is the case we should expect to find an increase in protein split products in the serum and an increase in the serum protease.

That this is actually the case will be observed from the text-figures and Table II. The only difference to be noted is the absence of any rise in the serum lipase which follows shock in anaphylaxis and peptone poisoning. If the adsorption that occurs when the kaolin reacts with the serum *in vitro* or *in vivo* were one of proteins, as Friedberger suggests, there would be no occasion for protein splitting. If, on the other hand, an adsorption of antiferment occurs (the antiferment being lipoidal is adsorbed most readily from the serum), we can easily understand that wherever such adsorption is in progress a localized area of antiferment deficiency must for the moment exist in which splitting can occur whenever ferments are present.

Just as in anaphylatoxin formation the matrix of the split products is to be found in the serum proteins, the kaolin acts merely as an agent which for an instant alters the normal ferment-antiferment balance and in this way brings about a protein intoxication.

Considering the fact that the first effect of acute shock is an increase of proteoses with practically no effect on the amino-acids, it would seem probable that the splitting takes place only to the higher stages. The splitting evidently differs from that occurring during anaphylactic shock (5), when we find first a decrease in proteoses and an increase in amino-acids, indicating a splitting through the peptone stage.

Whether other formed bodies, such as bacteria, ever bring about a toxic effect in this manner is of interest rather from a theoretical than a practical point of view, for the number of bacteria present in the serum would have to be very great to bring about marked changes. Bacteria do, however, readily adsorb the serum lipoids (6), and it seems probable that many of the phenomena which we classify under "*Serumfestigkeit*" are due to the changes so brought about.

CONCLUSIONS.

1. The intoxication produced by the intravenous injection of inert substances such as kaolin is due to protein split products derived from the serum proteins.

2. The kaolin acts as an adsorbing medium for the serum antiferment, bringing about an alteration in the ferment-antiferment balance.

3. The intoxication is accompanied by an increase in serum protease, and of proteoses.

4. The serum lipase, the amino-acids, and the total non-coagulable nitrogen show relatively little change.

5. The antiferment shows an initial increase, followed by a loss.

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THE EFFECT OF PROTEIN SPLIT PRODUCTS ON THE SERUM FERMENTS AND ANTIFERMENT.

Studies on Ferment Action. XXVI.

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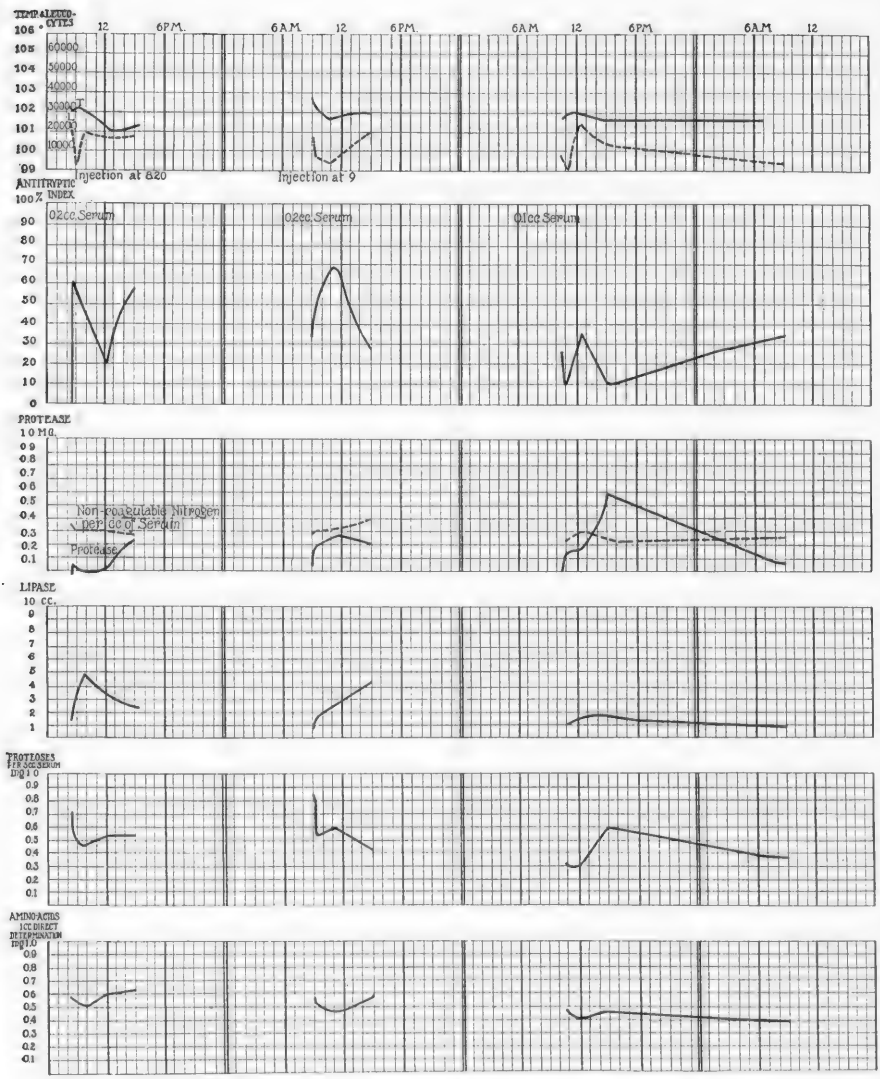
In previous papers we have discussed the ferment changes that occur in dogs during trypsin, anaphylactic, and kaolin shock, noting more particularly the relation of the serum protease to the antiferment and to the split products contained in the serum, together with changes in the lipase content. In the present paper we shall present the resulting ferment changes following the injection of various protein derivatives.

The fundamental ideas of Vaughan[1] relating to the toxicity of protein split products have been fully developed during the course of the last few years; the only element of uncertainty lies in the interpretation of certain phases of specificity of ferments which are supposed to split the native protein to toxic fragments. According to Vaughan's hypothesis, specific protease is produced capable of such function, a supposition which has received much support from the work of the Abderhalden school. There is, however, certain experimental evidence to the contrary, indicating that the specific element is not due to specific ferment action, but rather that the splitting that occurs is due to a non-specific ferment; that the split products are largely derived from the serum proteins and not from the injected antigen; while the element of specificity lies in the colloidal changes which bring about a lowering of the antiferment titer and in the rapid mobilization of the protease.

[1] Vaughan, V. C., Vaughan, V. C., Jr., and Vaughan, J. W., Protein Split Products in Relation to Immunity and Disease, Philadelphia and New York, 1913.



TEXT-FIG. 1. TEXT-FIG. 1 a. TEXT-FIG. 1 b.

TEXT-FIGS. 1, 1 a, AND 1 b. Serum changes following intravenous injections of (Text-fig. 1) proto-albumoses, (Text-fig. 1 a) primary proteoses, (Text-fig. 1 b) secondary proteoses.

While it is true that every protein when hydrolyzed will yield toxic substances, there is considerable variation in the degree of toxicity of the derivatives from different proteins. According to Baehr[2] this difference has some relation to the chemical configuration of certain components of the fragments. It has been found that of the various split products the primary proteoses are in general most toxic (Zunz, Jobling and Strouse, Zunz and György), the secondary proteoses being much less toxic. While shock produced by the split products is usually called peptone shock, the peptones, as a rule, are less toxic than the primary proteoses. The peptone preparation which we have used was, however, an exception to this statement, displaying an instantaneous and marked toxicity. It was prepared from dog muscle by the usual method.

The technique used in these experiments has been fully described previously, so that a repetition will be superfluous.

EXPERIMENTAL.

Effect of Primary Proteoses.

Dog 28.—Weight 7 kilos. 0.2 gm. of proto-albumoses (prepared by peptic digestion of dog serum) was injected intravenously at 9.45 a. m. The dog showed practically no evidence of malaise, although there was a slight primary fall in the leucocytes with a rise during the afternoon.

There was practically no change in the ferments and the antiferments; the non-coagulable nitrogen decreased slightly, although the amino nitrogen and the proteoses were unaltered.

Dog 70.—Weight 8.1 kilos. 0.5 gm. of proto-albumoses (from Witte's peptone) was injected intravenously at 8.20 a. m. The dog became nauseated and vomited almost immediately; it showed extreme prostration during the afternoon and died during the night (Text-fig. 1).

Dog 71.—Weight 6.6 kilos. 0.5 gm. of primary proteoses (from Witte's peptone) was injected intravenously at 9 a. m. Manifestations of intoxication were the same as in Dog 70. Death occurred during the night (Text-fig. 1 a).

In both these experiments there will be noted an immediate rise in the antiferment with a subsequent fall; a mobilization of protease more marked in Dog 71; a rather sharp rise in the serum lipase; and a distinct decrease in the serum proteoses and amino nitrogen. It is

[2] Baehr, G., *Proc. N. Y. Path. Soc.*, 1913, xiii, 151.

rather curious that the injection of 0.5 of a gram of proteoses should be followed by the decrease in the amount of the proteoses in the serum.

Effect of Secondary Proteoses.

Dog 73.—Weight 6 kilos. 0.5 gm. of secondary proteoses (from Witte's peptone) was injected at 10.50 a. m. The dog became somewhat nauseated but did not show the extreme prostration that characterized the two previous dogs; it showed no ill effects the following morning.

It will be observed (Text-fig. 1 b) that there occurred a well marked rise in serum protease, while the lipase was not affected. Proteoses were increased during the afternoon, but returned to normal the following morning. The amino nitrogen showed only a slight primary decrease.

Effect of Peptone.

Dog 74.—Weight 15 kilos. 0.25 gm. of muscle peptone injected at 9 a. m. (Text-fig. 2). The animal was immediately prostrated and was nauseated, after which it rapidly recovered and showed no further ill effects.

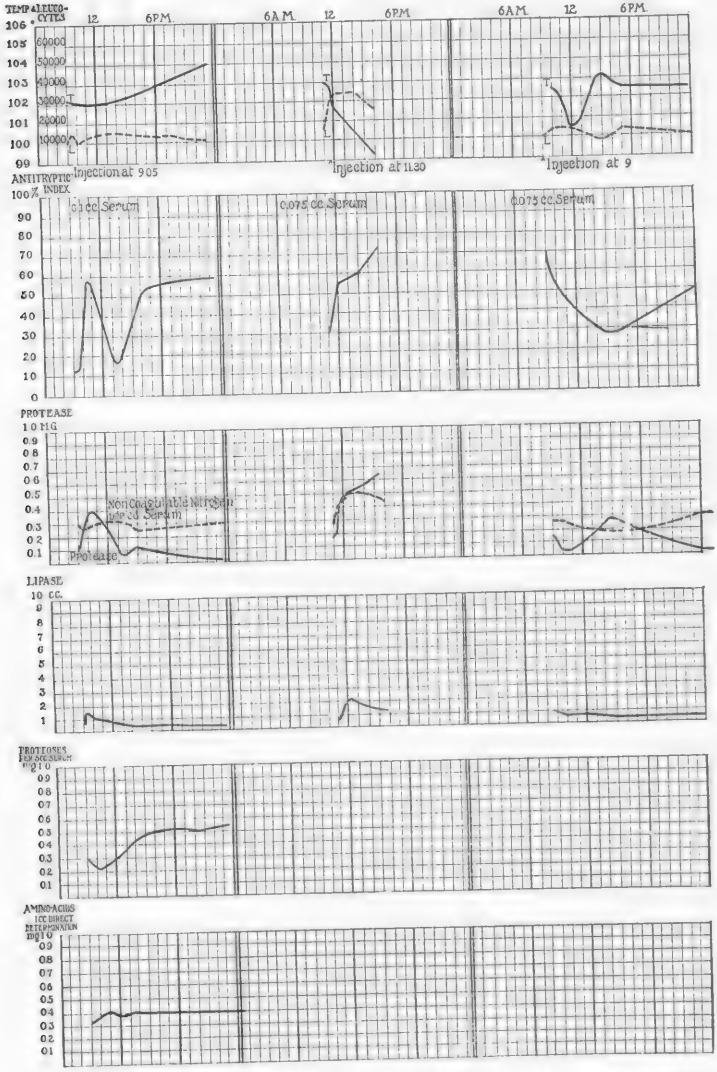
As will be observed from Text-fig. 2 there was almost an immediate rise in protease with a following decline. The antiferment showed rather a marked fluctuation, while the lipase showed only a slight rise with a fall in titer later. The proteoses were at first decreased but increased during the afternoon. The amino nitrogen showed only a slight increase.

Dog 43.—Weight 7 kilos. 0.17 gm. of muscle peptone was injected intravenously at 11.30 a. m. (Text-fig. 2 a). The animal became ill in a manner similar to Dog 74, and later developed a paresis of the hind legs. Died at 5 p. m.

In this animal the intoxication was more evident, both in its effect on the temperature and leucocyte curve, and in its effect on the serum ferments.

Dog 47.—Weight 4.5 kilos. This animal was given a very small dose (0.05 gm.) of muscle peptone intravenously at 9 a. m., without ill effects of any marked degree except an initial nausea and prostration (Text-fig. 2 b).

In contrast to the two previous dogs, however, there was a constant fall in the antiferment titer with a recovery the following day,



TEXT-FIG. 2. TEXT-FIG. 2 a. TEXT-FIG. 2 b.

TEXT-FIGS. 2, 2 a, AND 2 b. Serum changes following the intravenous injection of dog muscle peptone.

while the protease showed at first a decline and an increase during the afternoon. The lipase was not increased.

When introduced into the stomach or rectum (0.5 of a gram) the peptone was without toxic effect, and produced no alteration in the serum. When injected directly into the lumen of the small intestines, however, death resulted within five to ten minutes, the animal presenting the picture of profound shock.

CONCLUSIONS.

1. In dogs the toxic effect of primary proteoses is usually associated with the following serum changes: (a) an increase in serum antiferment, with a following fall in titer; (b) some increase in serum protease; (c) an increase in serum lipase; (d) a decrease in serum proteoses and amino nitrogen.

2. Secondary proteoses produce (a) less marked changes in the antiferment titer; (b) a marked increase in serum protease; (c) an increase in serum proteoses; (d) only a slight change in serum lipase; (e) a primary decrease in amino nitrogen.

3. The peptone which we have used (prepared from dog muscle) caused (a) a change in antiferment titer similar to that produced by the primary proteoses; (b) a marked increase in serum protease; (c) only a slight increase in serum lipase; (d) a primary decrease in proteoses, followed by an increase later; (e) an increase in amino-acids.

4. A very small dose of peptone resulted in a decrease in antiferment titer, together with a primary decrease in serum protease.

5. The peptone preparation was non-toxic when introduced into the stomach or rectum, while the intestinal injection was followed by an immediate intoxication.

We desire to express our appreciation to Messrs. T. B. and Paul Christian, who have materially assisted in various phases of this work.

THE EFFECT OF KILLED BACTERIA ON THE SERUM FERMENTS AND ANTIFERMENT.

Studies on Ferment Action. XXVII.

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(Received for publication, June 1, 1915.)

In a study of the serum reactions brought about by the injection of foreign substances, interest naturally centers on changes produced by the injection of various bacteria. That, apart from the well known immunity reactions, certain changes occur in the ferments of the serum when bacteria cause a reaction on the part of the host has become evident during the past few years, largely through a study of anaphylaxis and its correlation with immunological phenomena. The work of Vaughan, of Kraus, Pfeiffer, Friedberger, de Waele, Zunz, and many others, has emphasized the importance of protein intoxication, and, as a corollary, that of the ferments which bring about a lysis of the bacterial protein. In this way the study of the phenomena of infection and immunity has gradually centered about the idea that the hydrolyzed protein of the bacterium was responsible for the intoxication, while the unaltered protein was the cause of the immunity reaction.

More recently the subject has been somewhat confused rather than aided by the endeavor to demonstrate specific protease action and in this way possibly to connect the simple antibody reactions with the hydrolysis which results in an intoxication. In a recent paper (1) we have sought to demonstrate that the methods used and the results obtained in the course of such studies were seriously to be questioned; that the serum protease was not specific, but polyvalent; that in many instances the antigen was not the substrate

which yielded the split products, but that they were derived from the serum supposed to contain the specific ferment.

The older idea of Pfeiffer that the bacterium contained a preformed toxic substance which became evident on lysis has been gradually superseded, and the emphasis placed on the theory that the non-toxic proteins of the bacteria cell are hydrolyzed to toxic substances. There are, however, certain facts which have not been taken into consideration in the development of this hypothesis. They are as follows: (1) bacteriolysis has been confused with proteolysis, despite the fact that intact organisms are quite resistant to proteolytic ferments; (2) bacteria adsorb antiferment from the serum and in this way become even more resistant to proteolytic influence; (3) the serum contains an antiferment which prevents the splitting of native proteins, but does not prevent the splitting of the higher split products to amino-acids; (4) simple lysis of cells, as, for instance, by grinding and freezing, as Cole (2) has recently shown for pneumococci, may free substances which are toxic in a degree and manner similar to the protein split products derived in other ways; (5) the amount of preformed split products in the cell, which are set free by simple lysis, has been ignored.

In the following experiments we have used dried, killed organisms so that we might work with constant amounts and the factor of intravital multiplication be obviated.

The technique used in the determinations of the serum ferments, antiferment, and split products has been previously described (3).

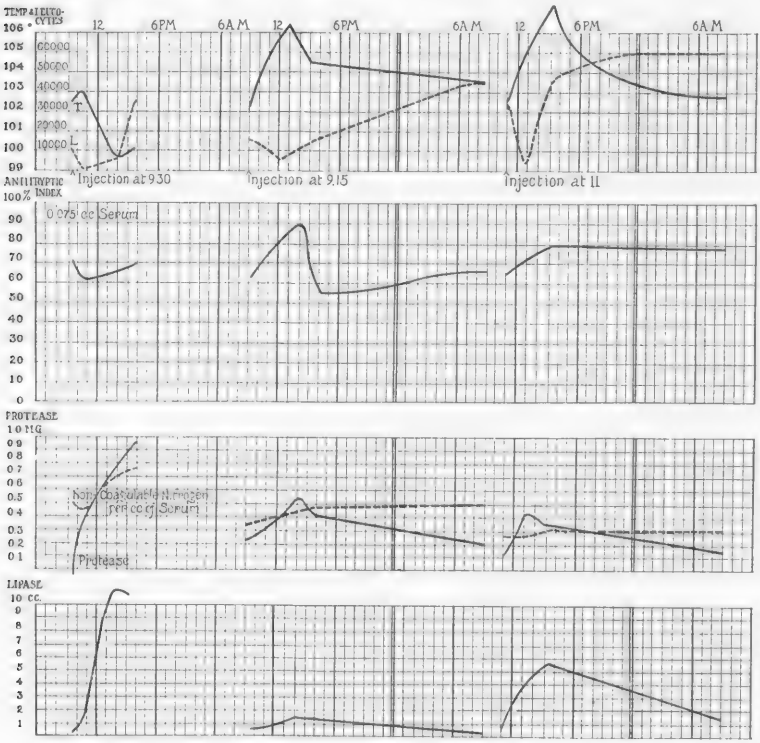
EXPERIMENTAL.

Typhoid Bacteria.

Dog 20.—Weight 7 kilos. 20 mg. of dried typhoid organisms were suspended in saline solution and injected intravenously at 9.30 a. m. (Text-fig. 1). The animal became ill shortly after the injection, was prostrated gradually, and died during the course of the night.

As will be observed from Text-fig. 1, the temperature, except for a slight initial rise, decreased during the afternoon, while the leucocyte curve at first declined and later increased. The antiferment showed little alteration. The most striking changes are noted

in the serum protease and lipase which increased to more than ten times their original titer. The non-coagulable nitrogen also increased after a slight initial decrease.



TEXT-FIG. 1. TEXT-FIG. 1 a. TEXT-FIG. 1 b.

TEXT-FIGS. 1, 1 a, AND 1 b. Serum changes following intravenous injection of typhoid bacilli; (Text-fig. 1), 20 mg., (Text-fig. 1 a), 10 mg., (Text-fig. 1 b), 10 mg., second injection.

The next dog was given a similar dose but in two injections.

Dog 38.—Weight 4 kilos. 10 mg. of dried typhoid bacteria were injected at 9 a. m.; a second injection of 10 mg. was made at 11 a. m Died at 5 p. m.

This experiment showed a similar increase in serum lipase and protease, although following the second injection there was no

further increase in protease, and a drop occurred. The antiferment showed no marked changes.

In the following dog a small dose was given after an interval of several days.

Dog 22.—Weight 6.5 kilos. 10 mg. of dried typhoid bacteria were injected at 9.15 a. m., Feb. 23, 1915 (Text-fig. 1 a). A second injection was made after 8 days, on Mar. 3, at 11 a. m. (Text-fig. 1 b).

The animal in both instances showed the usual evidences of illness. From Text-figs. 1 a and 1 b it will be observed, however, that, following the second injection, the mobilization of the protease was more rapid than after the first injection, and the rise in lipase was more marked. This is the condition usually occurring during sensitization (4). The antiferment showed less change the second time than the first, while the temperature and the leucocyte count showed evidence of even a more marked intoxication.

Various Organisms.

Dog 35.—Weight 4 kilos. 10 mg. of dried subtilis bacilli were injected at 9 a. m.

In this instance, while there was some change in the leucocyte count and a slight rise in temperature the only marked change occurred in the antiferment, which fell following the injection but increased later. The lipase increased slightly.

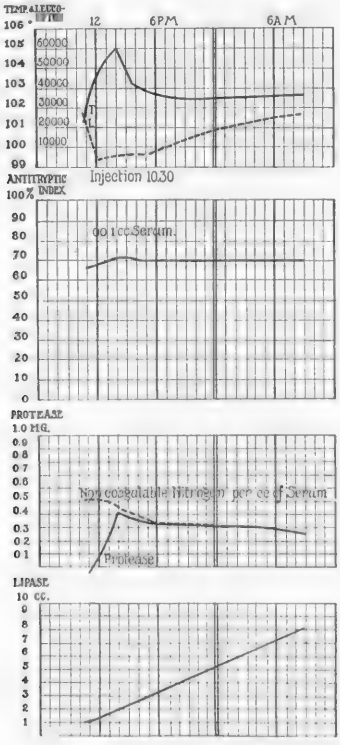
Dog 21.—Weight 4 kilos. 50 mg. of dried diphtheria bacilli were injected at 10.30 a. m. (Text-fig. 2).

The animal responded by a distinct rise in temperature and leucopenia. There was an immediate rise in protease, while the lipase increased progressively until the following morning. The antiferment was unaltered.

These two organisms differ widely in their resistance to tryptic digestion. *Bacillus subtilis* contains only a small amount of lipoids, autolyzes readily, and contains preformed a considerable amount of non-coagulable nitrogen. Diphtheria bacilli, on the other hand, contain considerable antiferment, are very resistant to tryptic digestion, and contain only a very small amount of non-coagulable nitrogen.

Typhoid organisms occupy rather an intermediate position (5). .It is interesting that the rise in lipase following the diphtheria injection occurs at a much later period than after typhoid injections.

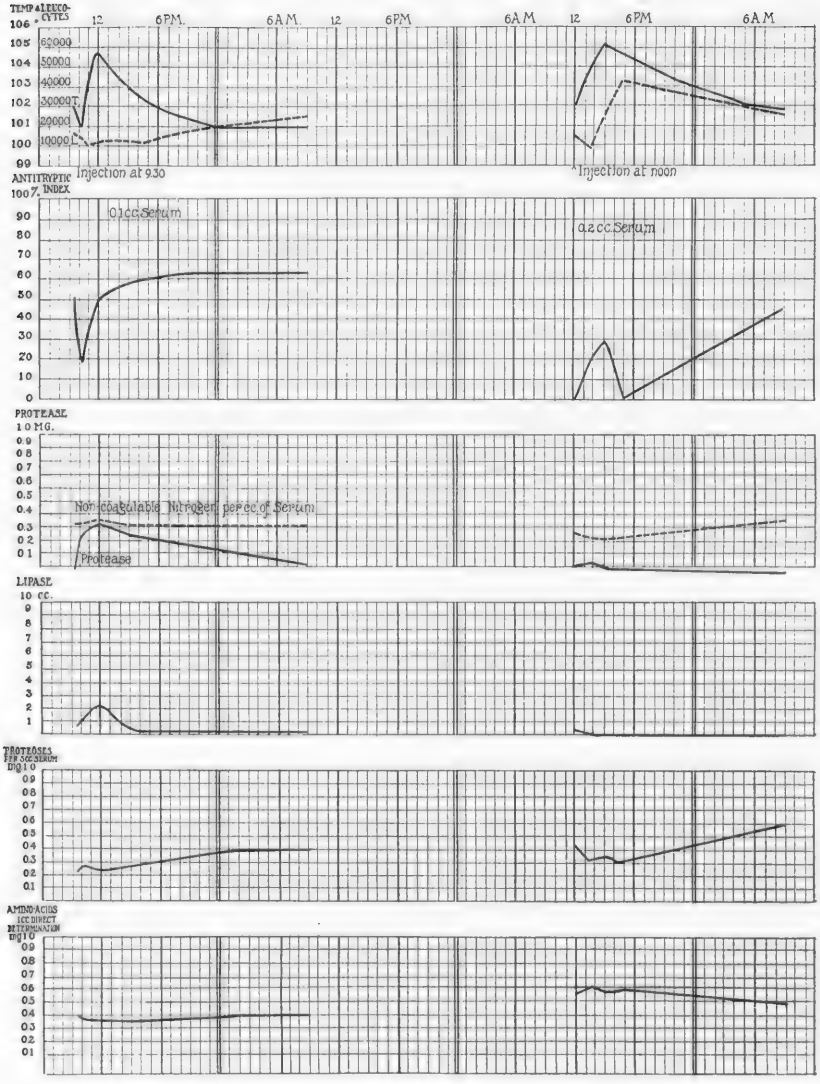
Dog 52.—Weight 4.5 kilos. 20 mg. of dried pneumococci (Neufeld strain) were injected at 11 a. m.



TEXT-FIG. 2. Serum changes following intravenous injection of diphtheria bacilli.

There resulted a rise in temperature to 105° F., and the leucocytes showed a primary rise. The serum, however, showed no marked changes; it is quite evident from this experiment that the temperature curve bears no direct relation to the ferment changes.

In the following experiments the serum proteoses and amino nitrogen have been determined in addition to the ferment changes.



TEXT-FIG. 3. TEXT-FIG. 3 a.

TEXT-FIGS. 3 AND 3 a. Serum changes following intravenous injection of (Text-fig. 3) staphylococci and (Text-fig. 3 a) tubercle bacilli.

Dog 76.—Weight 5.4 kilos. 10 mg. of typhoid bacteria were injected at 9 a. m. Death at 4 p. m.

The ferment changes were typical, the serum protease increasing from 0.05 to 0.7, the lipase from 1.2 cc. to 7 cc. at the time of death. There was a slight increase in serum proteoses while the amino nitrogen showed a primary decrease.

Dog 78.—Weight 6 kilos. 50 mg. of dried *Staphylococcus aureus* were injected at 9.30 a. m. The animal remained well and showed no evidence of the intestinal irritation always observed after injections of typhoid bacteria.

There will be observed, however, a sharp rise in temperature (Text-fig. 3), together with a marked fall in the antiferment titer. Protease and lipase increased, but returned to normal the following day. Serum proteoses increased, while the amino nitrogen showed a slight decrease.

Dog 79.—Weight 5.5 kilos. 100 mg. of dried tubercle bacilli (human) were injected at noon (Text-fig. 3 a). While the temperature and leucocyte curve rose considerably, the animal remained quite well and was free from nausea or gastro-intestinal symptoms.

The serum ferment remained unaltered, while the antiferment titer showed some changes. The proteoses at first decreased, but returned to slightly above normal the following day. The amino nitrogen showed a slight decrease after twenty-four hours.

DISCUSSION.

Incidental to the study of anaphylaxis (4), we have shown that the primary injection of protein, while it may be associated with malaise, a rise in temperature, and a leucopenia, is not associated with a change in protease, whereas the split products of proteins, as illustrated in the preceding paper,[1] may give rise to such a mobilization. It becomes quite evident, therefore, when we examine the effects produced, for instance, by typhoid bacteria, that the toxicity cannot be due to the effect of the native protein unless that protein is immediately hydrolyzed to its toxic components. We must assume either that such splitting takes place immediately on injection, or that

[1] Jobling, J. W., Petersen, W., and Eggstein, A. A., *Jour. Exper. Med.*, 1915, xxii, 597.

a simple lysis due to other causes sets free preformed toxic substances. Inasmuch as specific ferments are quite out of question at the time of the primary injection, we must acknowledge that whatever splitting may occur is due to a non-specific protease. Pfeiffer (6) has recently made observations following intoxication by burns and photodynamic agents, in which he determined the proteolytic strength of the serum with glycyltryptophane. He noted an increase following the intoxication, and discusses the possible relation of such ferments to the so called "*Abwehrfermente.*"

It is quite evident from the text-figures that if the development of toxicity depends on soluble substances derived from the killed organisms, these substances are at least in part preformed.

The relative toxicity bears some relation to the degree of resistance of the organism to the proteolytic ferment. This is noted with the tubercle bacillus (Text-fig. 3 a), which in large doses has caused a rise in temperature but no change in the ferments. The diphtheria organism (Text-fig. 2), which is also quite resistant, shows a maximum rise in lipase only after twenty-four hours, while the staphylococcus (Text-fig. 3), less resistant, shows a prompter effect. When we compare the toxicity of subtilis and typhoid bacilli, however, resistance to digestion cannot be the most important factor, for the subtilis bacillus is rather easily digested, at least as easily as the typhoid bacillus, whereas the toxic manifestations are quite out of proportion in the case of typhoid injection.

Under these circumstances we are rather of the opinion that proteolysis, in so far as it relates to the production of toxic substances from bacteria *in vivo,* is not to be emphasized as the sole agent in the mechanism, but rather that certain organisms contain preformed toxic split products which may be liberated during bacteriolysis, a reaction in which proteolysis has no part as yet demonstrable. On the contrary, it would seem warrantable to suppose that in the serum the protease might act rather as a detoxicating agent, in that it is able to split the toxic fragments to the non-toxic amino-acids. In this reaction the antiferment plays no part, for it seems to act only in inhibiting the splitting of the native proteins to toxic fragments. The mobilization of the protease, as of the lipase, occurs as a result of cellular injury, but is not necessarily associated with the febrile reaction.

CONCLUSIONS.

1. The intravenous injection of killed organisms is followed by the mobilization of a non-specific protease and lipase; the rapidity and extent of this reaction depend upon the toxicity of the organism and on the resistance of the organism to proteolysis.

2. The temperature and leucocytic curve bear no relation to the ferment changes.

3. The serum antiferment is usually increased after the injection.

4. Of the organisms studied, the typhoid bacilli produced the most marked ferment changes, and the tubercle bacilli the least.

5. The toxicity of the dried organisms cannot depend wholly upon proteolysis *in vivo,* but must depend in part on the preformed toxic substances liberated on lysis.

6. Serum protease should not be considered as the sole exciter of intoxication through the production of protein split products; it seems possible that its function may in part be one of detoxication.

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A STUDY OF THE CULTIVATION OF THE TUBERCLE BACILLUS DIRECTLY FROM THE SPUTUM BY THE METHOD OF PETROFF.

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The purpose of this paper is to confirm the work of Petroff[1] in a recent paper on the cultivation of the tubercle bacillus and to add a little from my own experience with the method.

Briefly, as outlined by Petroff, the sputum is mixed with a 3 per cent sodium hydroxide solution and incubated for a period of thirty minutes at 37° C. This is neutralized to sterile litmus paper with normal hydrochloric acid, centrifugalized, and the sediment inoculated on a veal-egg medium to which gentian violet in the dilution of 1 to 10,000 has been added.

From the standpoint of the general pathologist, since I had had variable results from antiformin, it seemed to me that if this method were available many avenues were opened up for the study of this organism in pure culture. It was therefore tried out in a series of twenty-five known positive sputa. At the same time other media, concerning which I hope to report later, were used as controls.

Many tubes were planted from each case, some with a platinum loop, and others by means of a sterile pipette. The latter is very satisfactory for inoculating a series of tubes rapidly with considerable amounts of material.

My results in the use of this method at first were discouraging, but, as often, the fault or faults were mine and not the method's.

[1] Petroff, S. A., A New and Rapid Method for the Isolation and Cultivation of Tubercle Bacilli Directly from the Sputum and Feces, *Jour. Exper. Med.*, 1915, xxi, 38.

Out of the twenty-five cases, from the last series of seven planted on twenty-one tubes I was able to secure ten tubes of gentian violet-egg-veal media with a pure culture of tubercle bacillus. Since my purpose was to test the method not so much for its rapidity as for its availability in isolating tubercle bacilli from generally contaminated material I think it may be stated that the method offers unlimited possibilities.

My failures may be grouped under three headings: drying, no growth, and contamination. Drying of this medium in the incubator and even at room temperature is very marked, so that it may be necessary to add a few cubic centimeters of sterile water to unused media kept only for a short time. Petroff also advises leaving the freshly inoculated tubes in the incubator for a few days until the moisture carried from the centrifuge tubes is absorbed and then paraffining the tubes. This has eliminated the question of drying. Secondly, no growth took place where known positive organisms existed. From my experience the reason for this probably was the neutralization of the sodium hydrate solution. As the neutral point is reached the liquid assumes a milky appearance. It would seem that it is better to stop just here, leaving the solution possibly a little alkaline in reaction. Thirdly, contamination is somewhat harder to explain. Contamination usually resulted in liquefaction of the medium. In my experience it occurred mostly in tubes without gentian violet, although some of the latter also suffered. Here (aside from the question of technique, for some of my contaminations, *c. g.,* with subtilis and spores, were due to errors in technique) there still remain some organisms which this method will not eliminate. These contaminations in some instances were due, in spite of care, to the frequent daily examinations of many of the tubes. The temperature of the incubator is an important point in successful cultivation; the maximum of range should be between 37° and 38° C.

As to the rapidity of growth much cannot be stated as yet. At the end of three weeks' incubation, however, well marked colonies appear which will continue to grow at room temperature. They start as small pin points, becoming multiple small, raised, grayish yellow points about 1 mm. in diameter at the end of two months.

Where they become confluent they preserve their original colonies, growing upon one another.

Microscopically these cultures bear out the statement, which is often made, that the tubercle bacillus has no morphology. It is acid-fast, resisting 30 per cent hydrochloric acid for at least thirty seconds; no pleochromatic forms are encountered. It occurs as rods, straight, curved, long, short, broad, thick, and thin; as small, large, and mostly single cocci; occasionally it resembles a streptococcus; forms similar to the diphtheria bacillus are seen, but larger, dumb-bell-shaped, clubbed, barred, and granular. The beaded appearance of the original organism from the sputum is conspicuous by its absence. Branching forms are encountered and, aside from the question of overlying, a Y-shape is the most frequent. The predominating type is a slightly curved, short, moderately thin, solid rod with denser granules at one or both ends.

CONCLUSIONS.

1. Probably in the majority of cases it is possible, with scrupulous technique, to isolate the tubercle bacillus from contaminated material such as the sputum, by the method outlined by Petroff.

2. It is necessary to use several tubes.

3. Neutralization, drying, temperature of the incubator, and contamination after the growth has started are important points to be noted in the process.

4. The colonies start as pin points, becoming larger and confluent, but still preserving individual groups of a dry appearance which simulate the medium closely in color.

5. The bacillus of tuberculosis in young culture is tinctorially acid-fast and of a polymorphous morphology.

EXPERIMENTAL ARTHRITIS IN THE RABBIT. A CONTRIBUTION TO THE PATHOGENY OF ARTHRITIS IN RHEUMATIC FEVER.

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(Received for publication, July 16, 1915.)

The significance of experimental arthritis in the rabbit has for many years been a matter of dispute. This may be attributed to the lack of data bearing upon the mechanism involved in the production of the lesions. It may be of interest to scrutinize the literature for facts which may relate to the conditions under which arthritis is, or fails to be, produced.

The first to produce arthritis in animals with pure cultures of streptococci was Loeffler (1), who published his results in 1884. Making cultural examinations of the exudate in the throats of patients with scarlet fever and diphtheria he isolated, besides the bacillus which now bears his name, several strains of streptococci, or micrococci, as he called them. Two of these strains, one from a fatal case of scarlet fever, the other from a fatal case of diphtheria, produced suppurative arthritis upon intravenous inoculation in 9 out of 12 rabbits. With Fehleisen's original strain of *Streptococcus erysipelatis* and by the same method he then produced a similar arthritis in 2 out of 3 rabbits. Smears from the pus in all cases showed abundant organisms and cultures made from 13 of them showed growth in 4, or 30 per cent—a high figure, considering the relatively crude methods employed. Most of the rabbits died.

Attention is called to the following points in Loeffler's work. There was between the time of injection and the development of arthritis an incubation period of 4 to 8 days, during which the animals were apparently normal. Arthritis followed only those injections which were given intravenously. Subcutaneous injections were followed by local infection usually ending in death without the evolution of joint lesions. The cultures, as shown by the mortality, were highly virulent. The infecting organism could be easily demonstrated in smears from the joint exudate and in a considerable percentage the cultures were also positive.

The streptococcus isolated by Wassermann (2) gave similar results, though it was probably not so virulent. The results are not reported in detail.

The diplococcus of Poynton and Paine (3) produced arthritis in 4 out of 9 rabbits injected (original report). The incubation period was 2 to 4 days. Two of the affected rabbits died on the 10th and 20th days, respectively, and two were killed. Only one recovered. Smears and cultures from the joint exudate were constantly positive. Here again the strain was evidently highly virulent.

Meyer (4) cultivated streptococci from tonsillar crypts of patients with rheumatic fever and produced arthritis in rabbits with these strains. The incubation period was 6 to 8 days and it was usually possible to demonstrate the organisms in numbers by smear and culture. Protocols and details are lacking.

Cole (5) in 1904 made a more careful study of experimental streptococcal arthritis. Using several strains isolated from widely varying conditions he produced with all a definite arthritis in rabbits,—also by intravenous inoculation. The organisms could usually be easily demonstrated in the joint exudate by smear and culture.

The most careful study of the one injection arthritis produced with virulent streptococci was made by Jackson (6). Using a highly virulent streptococcus isolated from the milk epidemic in Chicago she studied the joint reaction histologically at different periods after the injection. This report shows that 2 hours after injection streptococci could be found in the vessels of the periarticular tissues, that at 10 hours intravascular collections of leucocytes were present, while at 24 hours exudation and migration of leucocytes into the joint cavity had occurred. There is then a refractory period following the arrival of organisms at the joint during which no evident inflammatory reaction occurs, and this we may call the incubation period. It must be inferred that this reaction depends for its promptness and severity upon the virulence of the infecting organism. This is in accordance with the experiments of Dreyer (7) who found that the degree of reaction after injection of organisms into the joint of the rabbit is a good measure of their virulence. The point of most interest to us in Cole's paper is, however, the fact that two of his rabbits, inoculated with a less virulent strain, developed arthritis only after a second injection. In one of these rabbits smears and cultures were negative from all the affected joints except one and in this both smear and culture were positive. In several of the other animals spontaneous recovery occurred and in these Cole was able to provoke a relapse by another intravenous injection of the same organism. One attempt to cause a relapse with another strain of streptococcus failed.

Shaw (8) in 1904 and Schloss and Foster (9) in 1913 found that in monkeys arthritis occurred only after the second intravenous injection. Rothschild and Thalhimer (10) in 1913 note in their protocols that several of their rabbits with arthritis following intravenous inoculation of *Streptococcus mitis*, a slightly virulent organism, received five and six injections. In about one-third of these, cultures from the joint were positive.

It appears, then, that according to the virulence of the streptococcus used and possibly also according to the variety, two types of reaction may occur after intravenous inoculation. In one type, produced by streptococci of high virulence, the joint is attacked after the first injection. It is to be noted, however, that even in these instances a period of incubation is always observed. In this type of reaction cultures and smears from the affected joint are usually positive and the organisms are present in considerable numbers. In the second

type of reaction for which less virulent streptococci are used, no demonstrable joint lesion follows the first injection but a very definite arthritis does follow the second or, as will be shown, a still later injection. In other words the development of arthritis in these cases is conditioned probably upon some anterior process set up in the affected joint. In this type, cultures and smears from the joint frequently show no bacteria and, if bacteria are found, they are in much smaller numbers than in instances of the first type.

The number of definitely controlled and carefully reported experiments bearing upon the second type of reaction is quite small. A larger series is reported below.

In 1913 a Belgian investigator, Herry (11), reported a series of experiments which indicated the existence of a third type of reaction. In a remarkably large number of cases of rheumatic fever he was able to isolate a streptococcus from the blood or articular exudate. From this organism he obtained by a process of extraction with normal saline, desiccation, trituration, reextraction, and centrifugalization, an endotoxin soluble in water. By injection of this into the joint followed by an intravenous injection of the living organism 8 to 15 days later he was able to produce constantly in rabbits a definite arthritis in the joint originally treated. Cultures and smears from the joint were positive for about a week after the intravenous injection. Those experiments throw a clearer light upon the process of joint localization, but unfortunately they suffer from incompleteness.[1]

EXPERIMENTAL.

Experiments were therefore instituted with the object of treating joints with streptococci so that they might be more reactive to later intravenously injected streptococci and with the hope that arthritis might be made to localize in the joint so treated. It was thought that if this could be done the mechanism of joint localization might be partly explained.

[1] I have attempted without success to repeat Herry's experiments with extracts of the *S. mitis* used for the other experiments in this paper. Herry's method calls for the use of the supernatant fluid of a bacterial suspension after centrifugalization and it is probable that a certain number of organisms remained in this material. However, the author states that the same results may be obtained with endotoxin passed through a Chamberland filter. It should be stated that the subject of endotoxins in streptococci has been thoroughly studied and that present day opinion denies their existence. Herry's experiments are, however, illuminating.

A strain of *Streptococcus mitis seu viridans* was used for most of the experiments which was kindly lent by Dr. E. Libman of Mt. Sinai Hospital, New York. It was isolated by him from the blood of a patient with subacute endocarditis. It does not hemolyze blood, and grows on blood agar in small, discrete, dry, grayish colonies. It clouds glucose-ascitic agar. In broth it clumps and sinks to the bottom of the tube, leaving the medium clear. It is insoluble in rabbit bile and has no capsule. Since coming into our hands its pathogenicity for rabbits has been slight, a single intravenous inoculation of the contents of two 12 ounce Blake bottles of agar[2] failing to cause more than the slightest and most transient symptoms. This strain is designated No. 7. No. 4 is a similar organism from a similar source. No. 59 is a *Streptococcus viridans* obtained one year ago by Dr. Homer F. Swift from a case of pericarditis. It does not fall readily into any of the common groups of Andrewes and Horder. It is only slightly pathogenic for rabbits. Pn. I is a Type I pneumococcus isolated at the Hospital of The Rockefeller Institute. Pn. S. Afr. is a pneumococcus of Type IV isolated in South Africa from the Kaffir epidemic. The latter is not very pathogenic for rabbits. The *Bacillus typhosus* is a laboratory strain.

TABLE I.

Rabbits Receiving One Intravenous Injection of Streptococcus 7.

Rabbit No.	Amount injected.	Period of observation after injection.	Arthritis.	Remarks.
		days		
1	3 agar slants	49	0	
3	1 " slant	49	0	Intra-arterial.
5	$\frac{1}{3}$ Blake bottle	31	0	
6	$\frac{1}{4}$ " "	39	0	
7	" " "	13	0	
9	" " "	39	0	
19	1 agar slant	14	0	
38	" " "	37	0	Slight limp on 16th day. No swelling or other evidence of arthritis.

Total ..... 8 rabbits.
Arthritis ..... 0.

[2] The surface of the medium in the average Blake bottle is equal to that of 10 agar slant tubes.

Rabbits of medium size (of 1,200 to 1,800 grams' weight), usually females, were used. All injections were given in the ear vein except one or two into the femoral artery. The latter method was found to have no localizing effect in the corresponding leg.

Table I shows the effect of one injection, Table II of two injections, and Table III of three or more injections.

TABLE II.

Rabbits Receiving Two Intravenous Injections of Streptococcus 7.

Rabbit No.	Amount of 1st injection.	Interval between 1st and 2d injections.	Amount of 2d injection.	Arthritis.	Period of observation after 2d injection.
		days			*days*
8	$\frac{1}{4}$ Blake bottle	9	$\frac{1}{3}$ Blake bottle	0	45
36	$\frac{1}{2}$ agar slant	6	1 " "	0	20
K	$\frac{2}{3}$ " "	15	$\frac{1}{3}$ " "	0	1
L	" " "	15	" " "	0	13

Total ..... 4 rabbits.
Arthritis ..... 0.

It appears that with the streptococci used two sensitizing doses were needed before any of the rabbits developed arthritis. Smears from the joint exudate in a few cases showed a few streptococci and in three cases the cultures were positive. Cultures were made in most of the cases by planting the fluid in tall tubes of glucose-ascitic agar and in the others in ascitic bouillon, both methods appearing to be equally efficacious.

The fluid in different rabbits varied from a thick, practically purulent exudate to one showing only a moderate opalescence. All the exudates examined, with the exception of that from Rabbit 17, were viscid. All contained numerous polymorphonuclear leucocytes, and some large mononuclear lymphocytes and large endothelial cells. The last named and occasionally the other two types of cell frequently contained inclusions which were interpreted as phagocyted cocci. They were Gram-negative and somewhat larger than the cocci injected. Unaltered cocci were rarely seen within the cells. It is interesting to note that similar inclusions were described by Bosc and Carrieu (12) and have been seen by the writer in cells in exudate from human rheumatic fever.

TABLE III.

Rabbits Receiving Three or More Intravenous Injections of Streptococci.

Organism.	Rabbit No.	Amount of 1st injection.	Interval between 1st and 2d injections.	Amount of 2d injection.	Interval between 2d and 3d injections.	Amount of 3d injection.	Interval between 3d and 4th injections	Amount of 4th injection.	No. of injections.	Arthritis.	Joint affected.	Remarks.
			days		*days*		*days*					
Streptococcus 7	14	½ Blake bottle	9	½ Blake bottle	63	¼ Blake bottle	—	—	3	+	Both knees	Positive culture from joint fluid.
" "	15	" " "	9	" " "	63	" " "	—	—	3	+	" "	Positive culture from joint fluid.
" "	16	⅓ " "	1	⅓ " "	1	⅓ " "	33	⅓ Blake bottle	4	0	—	
" "	17	" " "	1	" " "	1	" " "	33	⅓ Blake bottle	4	+	Left wrist	Joint attacked 23 days after 3d injection. After recovery, a 4th injection caused a relapse.
" "	24	" " "	3	" " "	1	" " "	27	⅓ Blake bottle	4	0	—	
" "	25	" " "	3	" " "	1	" " "	27	⅓ Blake bottle	4	0	—	
" "	J	1/10 Blake bottle each day for 8 days.				Arthritis on 8th day			8	+	Both knees	
" 59	48	3 cc. broth culture	2	3 cc. broth culture	1	3 cc. broth culture	—	—	3	0	—	
" "	77	1 agar slant	1	1 agar slant	1	1 agar slant	—	—	3	+	Right shoulder	Positive culture from oint fluid.
" 4	49	4 cc. broth culture	3	" " "	1	" " "	—	—	3	0	—	

Total rabbits injected with Streptococcus 7..... 7. Arthritis..... 4.
" " " " " 59..... 2. " ..... 1.
" " " " " 4..... 1. " ..... 0.

The next series of experiments was made in an attempt to sensitize a joint with streptococci so that arthritis in that joint would follow a later intravenous injection of the same organism.

The left knee was used in all cases. The injections were made by the following technique:

A suspension of the organism was made by scraping the surface of an agar growth into sterile salt solution. In a few cases a broth culture was employed. The suspension was drawn into a syringe and the needle inserted through the patellar ligament just below the patella. It was carefully pushed proximally, avoiding the bone surfaces as much as possible until it was felt to slip forward easily. This indicated that the point of the needle was in the synovial pocket under the quadriceps. The suspension was slowly injected and a little of it made to flow back into the syringe by pressure over the lower part of the quadriceps (piston test). This was returned to the joint and the needle quickly withdrawn.

After a few trials it was possible to inject the joint without infecting any of the periarticular tissues, thus giving a sharply localized reaction. This procedure caused an arthritis which usually subsided in 2 to 4 weeks, the joint and its contents returning to their normal state as far as could be determined microscopically. Dead bacteria were rapidly phagocyted, while the living resisted destruction and removal for a longer time. In all but a few cases dead bacteria were used for sensitization.

At a varying period after the inflammation and its products had been shown by examination of the synovial fluid to have disappeared an intravenous injection of the same organism was given. The results are given in Table IV.

The exudate in all cases except Rabbit 84 failed to show growth and recognizable streptococci were seen in the smears only once. The picture seen in the smears had the same characteristics as in the cases of successive intravenous inoculation without direct sensitization. In Rabbit 58 a second intravenous injection after the reaction following the first had subsided was also followed by a transient articular reaction.

The effect of similar procedure with other bacteria was then investigated with the results shown in Table V.

The condition of susceptibility in a joint to intravenous injections of streptococci has been referred to as one of sensitization, but it

TABLE IV.

Rabbits Receiving an Intravenous Injection of Streptococcus 7 after Treatment of the Left Knee with the Same Organism.

Rabbit No.	Amount injected into knee.	Interval.	Amount injected intravenously.	Arthritis (gross signs).	Joint fluid.	
					Culture.	Smear.
		days				
26	$\frac{1}{6}$ Blake bottle, living	53	2 cc. broth culture	+	0	
34	1 cc.,* killed	28	1.5 cc. broth culture	+	0	P.n.l. +++ Inclusions +
35	" " living	28	1.5 cc. broth culture	+	0	P.n.l. +++ Inclusions +
45	0.2 " "	15	1 agar slant	+	0	P.n.l. ++ A few cocci.
53	0.5 " killed	60	2 cc.	+	0	P.n.l. ++ No bacteria.
58	0.2 " "	35	$\frac{1}{2}$ agar slant	+	0	P.n.l. +++
58†		65	1 " "	+	0	P.n.l. ++ No bacteria.
61	" " "	19	" " "	+	0	P.n.l. ++ No bacteria.
65	10 mg. dried cocci, precipitated with alcohol	17	" " "	+	0	P.n.l. +
66	10 mg. dried cocci, precipitated with alcohol	31	2 cc.	+	0	P.n.l. ++
84	0.2 cc., killed	36	$\frac{1}{2}$ Blake bottle	0§	+	No p.n.l. No inclusions.
111	" " "	14	" " "	+	0	P.n.l. ++++ Inclusions +
112	" " "	14	" " "	±	0	P.n.l. ++ Inclusions +
113	" " "	14	" " "	++	0	P.n.l. +++ Inclusions +
114	" " "	14	" " "	+	0	P.n.l. +++ Inclusions +
115	" " "	14	" " "	++	0	P.n.l. ++ Inclusions +

Total rabbits treated with Streptococcus 7..... 15. Arthritis..... 14.

*Unless otherwise specified, figures given indicate amounts of a bacterial suspension consisting of the contents of a Blake bottle agar growth at 24 hrs. taken up in 10 cc. of normal saline. The total is equivalent to the growth on 10 agar slants.

†65 days after the arthritis following the 1st intravenous injection had subsided a 2d intravenous injection was given and was likewise followed by marked inflammation of the joint.

§4 days later slight reddening and swelling of the joint appeared and polymorphonuclear leucocytes were found in the fluid, but the culture showed no growth.

TABLE V.

Rabbits Receiving Intravenous Injections of Streptococcus 59, Bacillus typhosus, or Pn. S. Afr. after Treatment of the Left Knee with the Homologous Organism.

Rabbit No.	Amount injected into knee.	Interval.	Amount injected intravenously.	Arthritis (gross signs).	Joint fluid. Culture.	Joint fluid. Smear.	Organism.
		days					
69	$\frac{1}{3}$ agar slant, killed	16	2 loops	0	−	−	*B. typhosus.*
91	0.5 cc., killed	29	$\frac{1}{2}$ agar slant	0	+	Some blood No excess cells	" "
92	" " "	31	$\frac{2}{3}$ " "	0	0	Normal, except that one cell shows inclusions	" "
93	" " "	31	" " "	0	0	Normal, except for one small clump of bacilli	" "
95	" " "	29	$\frac{1}{2}$ " "	0	0	Normal	" "
72	1 mg. bacteria sensitized by Gay's method	21	$\frac{1}{3}$ " "	±	0	Cells increased, 80% p.n.l.	S. 39
73	1 mg. bacteria sensitized by Gay's method	29	1 " "	+	+	No bacteria P.n.l. ++ Inclusions +	" "
83	0.2 cc., killed	35	$\frac{1}{2}$ Blake bottle	0	0	A few p.n.l. Inclusions +	" "
85	0.2 cc.	35	" " "	0	0	A few p.n.l. Inclusions +	" "
96	0.5 "	31	1 " "	0	0	Normal	Pn. S. Afr.
97	" "	31	1 broth tube	0	0	A few p.n.l. No inclusions	" " "

Total rabbits treated with B. typhosus ..... 5. Arthritis ..... 0.
" " " " Streptococcus 59 ..... 4. " ..... 2.
" " " " Pn. S. Afr. ..... 2. " ..... 0.

may be fairly asked whether any inflammation of the joint may not predispose to later joint affections. In other words, is the predisposing factor simply a non-specific inflammation or is it a specific sensitization? In order to answer this question a series of control experiments were made by injecting one sort of bacteria into the knee and following this with an intravenous injection of another sort (Table VI). By using two closely related strains of streptococci two doubtful (certainly very slight) reactions were obtained. By crossing with streptococcus and pneumococcus no reactions were obtained. It seems fair to conclude from these experiments that the reaction is due to a specific sensitization showing some evidence of a

TABLE VI.

Attempts at Cross-Sensitization.

Rabbit No.	Inoculation into knee. Amount.	Organism.	Interval.	Intravenous inoculation. Amount.	Organism.	Arthritis.	Joint fluid. Culture.	Joint fluid. Smear.	Remarks.
			days						
52	0.5 cc. susp., killed by heat	Streptococcus 7	69	$\frac{2}{3}$ agar slant, living	Streptococcus 59	?	o	Moderate number cells, 90% p.n.l. No bacteria	Slight or absent palpable swelling of knee.
67	10 mg. bacteria precipitated with alcohol	" "	24	$\frac{1}{4}$ " " "	Pn. II	o	o	Normal	No swelling.
76	0.2 cc. susp., killed by heat	" 59	21	$\frac{2}{3}$ " " "	Streptococcus 7	?	o	Moderate number cells, 90% p.n.l. No bacteria	Very doubtful palpable swelling of knee.
83	0.2 cc. susp., killed by heat	" 7	35	$\frac{1}{2}$ Blake bottle, living	" 59	o	o	Few cells. Rare p.n.l. No inclusions	No swelling or other gross evidence of arthritis.
84	0.2 cc. susp., killed by heat	" 59	35	" " " "	" 7	o	o	Normal. No p.n.l. No bacteria	No swelling or other gross evidence of arthritis.
106	0.3 cc. susp., killed by heat	Pn. S. Afr.	14	$\frac{1}{5}$ " " "	" "	o	o	Rare cells. 3 p.n.l. seen. No bacteria	No swelling or other gross evidence of arthritis.
107	0.3 cc. susp., killed by heat	" " "	14	" " " "	" "	o	o	Rare cells. 1 p.n.l. seen. No bacteria	No swelling or other gross evidence of arthritis.
108	0.3 cc. susp., killed by heat	" " "	14	" " " "	" "	o	o	No excess cells No bacteria	No swelling or other gross evidence of arthritis.
109	0.3 cc. susp., killed by heat	" " "	14	" " " "	" "	o	o	Very few cells. No p.n.l. No bacteria	No swelling or other gross evidence of arthritis.
110	0.3 cc. susp., killed by heat	" " "	14	" " " "	" "	o	o	A few cells. About 10% p.n.l. No bacteria	No swelling or other gross evidence of arthritis.

Total rabbits tested for heterologous sensitization 10
" " showing joint reaction ..... 2 (doubtful).

group specificity comparable with the group agglutinations of certain bacteria.

Pathological Anatomy.

The arthritis following intravenous inoculation alone and that following intravenous inoculation after local sensitization presented the same gross and microscopic picture. An exception must be made in the case of Rabbits 14 and 15, in which a chronic arthritis with the continued presence of demonstrable living streptococci in the exudate followed the last injection. Here erosions in the articular cartilages were found. In the other rabbits the joint at autopsy showed the following changes: The synovial surface was moderately congested, the villi usually more so than the other parts. An excess of stringy fluid was usually present. The capsule was somewhat swollen. In cases which had reacted severely there was occasionally found after inflammation a small amount of inspissated exudate in the upper angle of the joint cavity. Sections showed a marked leucocytic infiltration of the villi and to a slighter degree of the subendothelial layer of the capsule. Examination of the adjacent cartilage and bone failed to show any noticeable change. Examination of the articular and periarticular tissues for bacteria by the Gram stain did not reveal the presence of organisms after a careful search. The vessels of the capsule and of the villi were moderately distended but no thrombi were seen. The reaction as seen under the microscope appeared to be mainly in the villi with the synovial membrane also playing a part.

DISCUSSION.

It is believed that the above experiments may throw some light upon the mechanism of acute arthritis. It seems to be established that the first attack of a highly virulent streptococcus can cause an arthritis and that the arthritis so produced is usually of a severe type with an exudate containing large numbers of viable organisms.

On the other hand, streptococci of lower virulence are frequently not able to produce arthritis at the first attack but at this time prepare the way for such an effect in a later attack.

It seems to be clearly proved that this preparatory or sensitizing process is, within narrow limits, a strictly specific one; *i. e.,* the organism used for the exciting, intravenous injection must be the same as that used for the sensitizing, intra-articular injection, else the reaction fails to occur.

It further appears that this preparation may be made by the introduction into the joint of the organism, living or dead, and it seems fair to conclude that the arthritis produced by successive intravenous injections results from a similar process; *i. e.,* a preliminary deposit of organisms in the joint leading to sensitization but not to gross inflammatory lesions, the latter resulting from a subsequent deposition of organisms. It is also shown that this reaction in a sensitized joint may be repeated several times in the same animal.

A close analogy between this reaction and the relapses in rheumatic fever can readily be drawn. Relapses are so common in this disease as to be one of its distinguishing characteristics. Thus in the figures given by Mosler and Valentin (13) 60 out of the 142 cases studied were suffering from their second or a later attack at the time of admission to the hospital. Cole produced 8 successive attacks, each followed by recovery, in a single rabbit.

Without assuming the specificity of any one organism the conception of relapsing arthritis as the effect of a virus upon an homologously sensitized joint may be fairly applied to the relapsing cases of human rheumatic fever.

The reaction of a sensitized joint in rabbits inoculated intravenously may properly be designated as an induced relapse and it will be seen from the protocol of Rabbit 17 that a second relapse can be induced by a second intravenous injection. From the ease and constancy with which lesions can be induced in sensitized joints by the method above outlined it is strongly suggested that the relapses, if not the primary arthritis, in rheumatic fever result from a virus reinvading the blood stream and provoking a reaction in a previously sensitized locus.[3]

[3] It may not be too wide a step to pass from the consideration of the effects induced in the joints to the phenomena observed in the endocardium and possibly even the myocardium in rheumatic fever. That they too are of relapsing nature is admitted; and that they also result from sensitization may be suggested.

In respect to the primary attack an analogy may be drawn from the fact above noted, that in rabbits and monkeys there is needed either a period of incubation, or, in most cases, a series of injections of the exciting organism before the joint is attacked. This fact suggests the hypothesis that some degree of sensitization, or of heightened activity on the part of the fixed cells is necessary before a definite and marked tissue reaction occurs.

It is not desired to state any opinion as to the identity of the organism causing rheumatic fever. In view of the fact that several different organisms can cause arthritis in the rabbit, that several different organisms can cause arthritis in man, and that the clinical manifestations of rheumatic fever vary widely it may well be that no one organism is constantly at fault. Nevertheless the streptococcus, and in most cases a member of the *viridans* group, has been the one most often found when cultures are positive. Further, this organism shows greater and more constant arthrotropic properties than any other now known. The preponderance of the evidence now available, therefore, is with the streptococcus. At this point the case must rest until further proof is offered.

SUMMARY.

By a process of sensitization described it was found possible to cause arthritis in rabbits constantly after one intravenous injection of the streptococcus.

This reaction is specific.

By intravenous inoculation, without previous sensitization, of the streptococcus used in these experiments it was possible to cause arthritis in rabbits only after three or more injections.

An analogy is suggested between the arthritis induced by sensitization and the relapses in human rheumatic fever.

A further analogy is suggested between the development in rabbits of arthritis after repeated intravenous injections and the development of the primary lesion in human rheumatic fever.

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THE ACTION OF THE LETHAL DOSE OF STROPHANTHIN IN NORMAL ANIMALS AND IN ANIMALS INFECTED WITH PNEUMONIA.

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PLATES 74 TO 76.

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The following experiments were undertaken for the purpose of ascertaining whether the action of a digitalis body when administered to animals suffering from pneumonia differed from its action when no infection or fever was present. That there is a difference of opinion regarding the action of the drug when given in acute infections is shown in the practice of clinicians.

For example, Mackenzie (1) doubts its value and says that digitalis "is often used when patients are dying of some grave affection, as pneumonia, as a last resort, probably more for the purpose of doing something than with expectation of great benefit. I have never seen much good follow the administration of digitalis in acute febrile states." Gibson (2) in his article on acute pneumonia also says, regarding the use of digitalis, that in the worst cases of pneumococcal poisoning the heart absolutely refuses to respond. On the other hand, in Krehl's clinic (3) digitalis is used in all cases of pneumonia with the belief that it has a beneficial effect.

On account of this difference of opinion, Gunn (4) recently performed experiments with the view of determining what influence the height of the temperature has on the action of digitalis. In his experiments he perfused excised rabbits' hearts with solutions of strophanthin at different temperatures. The perfusion was carried to the point of arrest of the heart's action. By this procedure Gunn demonstrated that with variations in temperature ranging from 28° to 41° C. strophanthin acts more quickly on the isolated heart as the temperature is raised. The conditions in Gunn's experiments are not analogous to those in pneumonia, since in the presence of this infection toxemia accompanies the increased temperature.

In the experiments now reported the attempt was made to duplicate the usual human conditions as nearly as possible. A digitalis

body was administered to animals suffering from an acute pneumonic infection and the effect compared with that obtained when the drug was given to healthy animals of the same species. A comparison of the results in the two groups we believe should yield information regarding the influence of infection on the action of the drug.

Methods.

The animals employed were cats and dogs. In each species one group was infected and another group was used as a control. The animals were infected by the method of intrabronchial insufflation described by Lamar and Meltzer (5). The organism used was an eighteen to twenty-four hour old broth culture of virulent pneumococci[1] belonging either to Type I or II (6), and was obtained from the laboratory of the Hospital. Of these cultures the cats received from 10 to 20 cc., and the dogs from 20 to 30 cc. The temperature of the animals was taken daily for several days before infection and also during the course of the disease.

The digitalis body employed was Thoms crystalline g-strophanthin (Merck). An alcoholic stock solution of the drug was made and from this an aqueous solution was prepared for immediate use. In the experiments on cats the aqueous solution was of such a strength that 10 cc. of solution contained 0.1 mg. of strophanthin. For the dogs a solution double this strength was employed. In all experiments the strophanthin was given intravenously. The animal was lightly anesthetized with ether, the femoral vein exposed, and a small glass cannula was inserted. This was connected with a burette containing the solution. The anesthetic was not continued beyond the time necessary for the insertion of the cannula. In injecting the strophanthin, an endeavor was made to maintain a uniform rate of flow. A lethal dose was given to each animal in approximately one hour, unless the animal died before the time required for administering the lethal dose had elapsed. The amount of strophanthin injected differed slightly in cats and dogs. In a large series of experiments on cats, Hatcher and Brody (7) and, later, Eggleston (8) found the lethal dose of strophanthin to be 0.1 mg. per kilo of

[1] The virulence was such that 0.000001 cc. was fatal in white mice.

body weight. In the present experiments on cats this amount was injected in both the control and the infected animals. In the dogs it was necessary first to estimate the minimal lethal dose (m.l.d.) in normal animals; it was found to be 0.12 mg. per kilo of body weight. Autopsies were performed on all the infected cats and on all cats in the control group which succumbed to the lethal dose of strophanthin. All the dogs, whether normal or infected, were autopsied. In a number of cats and dogs electrocardiograms were taken before infection and also just before injecting strophanthin. A series was also made during the period of injection.

TABLE I.

Normal Cats Injected with Strophanthin.

Date (1914).	Cat.	Sex.	Weight at time of injection.	Strophanthin injected.	Amount of m. l. d. + or −.	Result.	Duration of experiment.
			kilos	*mg.*	*mg.*		*min.*
Nov. 3	A	F.	2,200	0.22	L*	D†	52
" 3	B	M.	2,400	0.24	L	D	60
" 3	C	F.	2,100	0.21	L	D	110
" 7	E	M.	3,150	0.315	L	S	—
" 7	F	M.	3.000	0.3	L	D	50
" 7	G	F.	2,500	0.25	L	D	59
" 7	H	F.	2,850	0.285	L	D	70
" 10	I	F.	2,000	0.2	L	S	—
" 10	J	M.	3,650	0.365	L	D	65
" 10	K	F.	2,300	0.23	L	S	—
" 14	L	F.	2,925	0.253	L−0.0395	D	48
" 14	M	F.	4,050	0.405	L	D	63
" 14	N	M.	1,700	0.17	L	S	—
" 20	P	M.	3,300	0.33	L	D	60
" 20	Q	F.	1,975	0.187	L−0.01	D	27
" 20	R	F.	1,700	0.17	L	S	—
" 20	S	M.	1,800	0.18	L	S	—
" 25	T	F.	3,050	0.305	L	D	78
" 25	U	M.	2,800	0.28	L	S	—
" 25	V	M.	2,425	0.2425	L	S	—
" 25	W	M.	2,250	0.225	L	S	—

Total = 21.
Died = 12 = 57.1 per cent.
Survived = 9 = 42.9 per cent.

* In the tables L represents the estimated lethal dose. L + or — indicates the amounts more or less than the estimated lethal dose.

† D = died; S = survived.

In the experiments on cats, three groups were studied: a control group and two groups of infected animals. The control group con-

sisted of normal, uninfected animals, each of which received 0.1 mg. of strophanthin per kilo of body weight. This is the amount reported by Hatcher and Brody as the minimal lethal dose. The second group consisted of infected animals. They were otherwise untreated, and served the purpose of observing the course of the disease. The animals in the third group were also infected. Each of these received an amount of strophanthin equal to that given to the first group.

Group I (Table I) formed the control series of experiments. This group consisted of twenty-one cats, all of which were apparently normal and healthy. All received the estimated lethal dose of strophanthin (0.1 mg. per kilo of body weight) in the manner described. Of the twenty-one cats, twelve, or 57.1 per cent, died, while nine, or 42.9 per cent, survived. In the cats that died, the time that elapsed from the commencement of the injection until death occurred ranged from 27 to 110 minutes, the average time being 62 minutes. All the cats received the estimated lethal dose with the exception of animals L and Q. These two died when slightly less than the estimated amount had been injected. The low mortality in this group is a subject which will be discussed later.

TABLE II.

Cats Infected with Pneumococci, but Receiving No Strophanthin.

Date of infection (1914).	No. of cat.	Sex.	Type of organism.	Highest temperature before and after infection.	Result.
				° C.	
Nov. 23	34	F.	II	38.5–38.8	D Nov. 26.
" 23	35	M.	II	38.4–39.6	S
" 23	36	M.	II	37.7–39.6	D " 27.
" 23	37	M.	II	38.2–38.6	S
" 24	38*	F.	I	38.2–38.8	D Dec. 14.
" 24	39	F.	I	38.6–39.3	S
" 24	40	M.	I	39.0–39.0	S
" 24	41	F.	I	38.4–39.2	S
" 27	42	M.	II	38.2–39.4	S
" 27	43	M.	II	39.0–39.6	S
" 27	44	M.	II	39.0–39.4	S
" 27	45*	F.	II	37.8–39.0	D " 14.

Died = 16.6 per cent.

* The two cats, Nos. 38 and 45, which died three weeks after infection, evidently not from pneumonia, but from some intercurrent infection, are not considered in estimating the mortality for the series.

Group II (Table II) comprised twelve cats, each of which was infected by an intrabronchial insufflation of pneumococcus culture. Six of the animals developed very severe symptoms. The other six were only moderately ill. Of the former, one died on the third and one on the fourth day. The symptoms of the disease appeared eight to twelve hours after infection. The animals became listless and prostrated. Almost all showed a rise in temperature ranging from a fraction of a degree to 3.6° C. The symptoms of the disease apparently reached their height on about the second or third day after infection. At this point convalescence set in. Practically all the animals lost weight.

Group III (Table III) consisted of forty-three cats, all of which were infected. In seven, the culture used was obtained at autopsy from the heart's blood of infected cats; the rest were infected with organisms obtained in the manner already described. The symptoms of disease corresponded to those described under Group II. Ten cats failed to show a rise in temperature, but the temperature was not taken sooner than twenty-four hours after infection, and it is possible that, had readings been made at an earlier period, a rise might have been observed. A few animals which showed only a very slight reaction to the infecting organisms after the first insufflation, were reinfected. Strophanthin was given as nearly as possible at the height of infection; that is to say, on the second or third day, according to the observations made in Group II. Following the injection, twenty-three cats, or 53.5 per cent, died, and twenty, or 46.5 per cent, survived. The cats that died during or following the injection did so in from 25 to 108 minutes, the average time being 62 minutes. Nine cats succumbed before the injection was completed. They received slightly less than the estimated lethal dose. Three cats, Nos. 17, 48, and 53, through an error in calculation, received slightly more than the estimated lethal dose, but as these cats all survived the error does not affect the results.

A comparison of Groups I and III, the animals in both of which received strophanthin, shows that the mortality in each series, following the injection of the adopted lethal dose of the drug, was practically the same; *viz.*, 57.1 and 53.5 per cent. The course of the disease in the infected animals, however, was short; in many in-

TABLE III.

Cats Infected with Pneumococci, and Later Injected with Strophanthin.

Date (1914).	No. of cat.	Sex	Type of organism.	Weight on day of injection.	Strophanthin injected.	Amount of m. l. d. + or −.	Result.	Duration of experiment.	Highest temperature before and after infection.
				gm.	*mg.*	*mg.*		*min.*	° *C.*
Oct. 26	1	F.	I	2,250	0.203	L −0.022	D	47	37.8–39.2
" 26	2	F.	I	2,000	0.20	L	S	—	36.2–39.8
" 28	3	M.	II	1,750	0.175	L	D	57	——
" 28	4	F.	II	2,550	0.245	L −0.01	D	62	——
" 29	6	F.	I	2,000	0.15	L −0.051	D	45	36.7–39.0
" 29	7	F.	I	1,300	0.13	L	S	—	36.0–38.0
" 30	9	—	II	2,700	0.27	L	S	—	37.0–38.1
" 30	10	F.	I	2,250	0.225	L	S	—	37.2–37.8
Nov. 2	11	F.	II C*	1,500	0.15	L	D	57	37.8–37.8
" 11	12	M.	II C I	3,100	0.31	L	D	66	38.1–38.6
" 11	13	F.	II C I	1,875	0.1875	L	S	—	38.6–40.0
" 11	14	M.	I	2,575	0.2575	L	D	76	38.8–39.0
" 12	16	F.	II C	3,000	0.30	L	S	—	39.0–39.4
" 12	17	M.	II C	2,200	0.225	L +0.005	S	—	38.8–38.8
" 12	18	M.	II C	2,250	0.225	L	D	88	39.2–40.0
" 13	19	M.	II	3,600	0.3595	L −	S	—	39.0–39.2
" 13	22	M.	II	3,325	0.254	L −0.0785	D	42	39.2–39.0
" 18	23	F.	I	2,425	0.2425	L	D	80	39.1–38.6
" 18	24	M.	I	3,500	0.319	L −0.031	D	62	39.0–38.2
" 18	26	M.	I	2,475	0.2475	L	D	60	38.4–39.4
" 19	15	F.	II C Nov. 10 I " 17	2,900	0.183	L −0.107	D	25	38.6–39.6
" 19	27	M.	I	1,700	0.17	L	D	92	39.0–39.8
" 19	28	F.	I	2,225	0.2225	L	D	73	38.4–37.8
" 19	29	F.	I	2,350	0.235	L	D	108	38.2–39.0
" 23	30	F.	II	3,050	0.275	L −0.03	D	48	38.1–38.2
" 23	31	M.	II	3,750	0.375	L	S	—	38.0–40.6
" 23	32	F.	II	2,500	0.25	L	S	—	38.2–39.0
" 23	33	F.	II	2,575	0.2575	L	S	—	38.8–39.6
" 30	48	F.	II	2,700	0.271	L +0.001	S	—	38.8–40.6
Dec. 2	50	F.	II	3,000	0.30	L	D	60	38.5–39.0
" 2	51	F.	II	2,500	0.25	L	S	—	39.0–39.8
" 2	52	F.	II	2,900	0.29	L	D	60	37.5–38.3
" 2	53	M.	II	3,325	0.354	L +0.0215	S	—	38.2–39.3
" 3	55	M.	II	3,850	0.385	L	S	—	39.2–39.0
" 3	56	M.	II	2,950	0.295	L	S	—	38.2–41.2
" 4	57	F.	II	3,100	0.31	L	D	65	38.2–38.8
" 7	62	F.	II	3,000	0.3	L	S	—	38.2–39.4
" 8	63	M.	II	3,400	0.34	L	S	—	38.0–40.0
" 9	64	M.	I	2,500	0.25	L	S	—	37.6–39.2
" 10	65	F.	I	1,550	0.155	L	S	—	38.2–36.6
" 11	66	F.	I	3,050	0.265	L −0.04	D	63	38.0–38.0
" 15	60	F.	II	2,000	0.13	L −0.07	D	37	38.6–38.4
" 18	59	F.	II	1,600	0.16	L	D	46	38.4–39.4

Total = 43.
Died = 23 = 53.5 per cent.
Survived = 20 = 46.5 per cent.

* C indicates that the culture used was obtained from the heart's blood of a cat previously infected.

stances the animals were not seriously ill, and a number failed to show any increase in temperature, or showed an increase which was comparatively slight. It was considered inadvisable, therefore, to draw conclusions from these experiments. The attempt was accordingly made to enlarge our experience by performing experiments in dogs.

The Action of Strophanthin in Normal and Infected Dogs.

The experiments on dogs were undertaken with the expectation that they would show a greater degree of reaction to infection than cats. No standardization of strophanthin in dogs has been made (in so far as could be ascertained), so that it was necessary first to determine the lethal dose of the drug. Experiments were, therefore, carried out on ten dogs (Table IV) to determine the minimum lethal

TABLE IV.

Dogs Injected with Strophanthin, To Estimate the Lethal Dose.

Date (1915).	Dog.	Sex.	Weight at time of injection.	Total strophanthin injected.	Strophanthin injected per kilo of body weight.	Duration of experiment.	Deviation from average lethal dose.
			gm.	*mg.*	*mg.*	*min.*	*per cent*
Jan. 20	A	F.	8,000	0.98	0.122	47	2
" 21	B	F.	5,100	0.57	0.111	42	10
" 22	C	M.	7,350	1.07	0.145	110	16
" 25	D	F.	9,000	0.9	0.1	65	17
" 28	E	M.	5,700	0.704	0.123	53	1
Feb. 10	F	F.	5,750	0.75	0.130	95	5
" 10	G	M.	5,000	0.59	0.118	57	5
" 15	H	F.	5,300	0.606	0.114	68	8
" 15	I	F.	4,150	0.55	0.132	90	6
" 16	J	F.	4,750	0.71	0.149	93	20

Average dose per kilo = 0.124 mg.

dose. Before injection the animals were observed for a time under uniform conditions and all were apparently normal and healthy. The injections were made during the months of January and February. The females used were neither pregnant nor lactating. The animals were not fed for a period of eighteen hours preceding the experiment. This short fast was enforced so that the presence of food in the stomach should not influence the weight of the animal, and so that vomiting during the preliminary anesthesia might be prevented.

A fresh alcoholic solution of strophanthin was prepared and the aqueous solution made from this was of a strength such that 5 cc. represented 0.1 mg. of strophanthin. The solution was tested on three cats and was found to have the same effect as had the first stock solution. The lethal dose for these three animals was 0.108, 0.127, and 0.107 mg. per kilo of body weight, respectively.

The injection of strophanthin in dogs was made in a manner identical with that described for cats. The lethal dose for the ten dogs used was found to average 0.124 mg. per kilo of body weight. Death occurred in approximately 60 minutes. The dose ranged from 0.1 to 0.149 mg. per kilo of body weight. Of the ten dogs, seven showed a deviation of 10 per cent or less from the average m. l. d. The remaining three dogs, C, D, and J, gave respectively a deviation of 16 per cent above, 17 per cent below, and 20 per cent above the average. If the three dogs showing the greatest variation be deducted from the series, the result is not materially altered and the m. l. d. still remains 0.12 mg.

TABLE V.

Dogs Infected with Pneumococci and Later Injected with Strophanthin.

Date (1915).	No. of dog	Sex.	Type of organism.	Weight before infection.	Weight at time of injection.	Total strophanthin injected.	Strophanthin injected per kilo of body weight.	Duration of experiment.	Deviation from average lethal dose.	Highest temperature before and after infection.
				gm.	*gm.*	*mg.*	*mg.*	*min.*	*per cent*	°C.
Jan. 15	1	F.	II	9,000	7,900	1.237	0.137	108	10	38.8–39.2
" 18	3	F.	II	8,150	7,300	1.105	0.151	85	20	39.0–40.9
" 26	4	M.	II	9,400	8,800	1.146	0.130	78	4	39.8–39.5
Feb. 1	5	M.	II	8,500	7,800	0.82	0.105	55	16	39.8–39.5
" 3	6	M.	II	9,000	7,900	0.88	0.111	73	11	39.6–39.2
" 4	8	F.	II	8,300	8,050	1.01	0.125	95	0	39.8–39.6
" 17	10	F.	II	5,800	5,750	0.68	0.118	73	6	38.2–40.4
" 19	11	M.	II	6,350	6,150	0.614	0.0998	55	20	38.8–39.8
" 24	12	M.	I	8,600	7,500	1.08	0.144	115	15	39.0–39.8
" 26	14	M.	I	8,500	8,250	1.1	0.133	90	6	38.5–41.5

Average lethal dose per kilo = 0.125 mg.

A series of ten experiments was next carried out to ascertain the lethal dose in pneumonic dogs (Table V). These animals were infected by the method described. In the reaction to the infection, the dogs displayed symptoms similar to those described for the cats. They became listless and usually refused to eat. The respirations

appeared to be somewhat labored and were increased in rate. In all the dogs there was a loss in weight, amounting in two to more than 1,000 grams. The temperature following infection usually rose. In seven dogs the increase ranged from a fraction of a degree to 3° C. Three dogs, however, failed to show an increase, but in these the temperature was not taken until twenty-four hours after infection with pneumococci. The injection of strophanthin was made during the second twenty-four hours following infection because this was the time when the animals were most severely ill. Lamar and Meltzer (5) and, later, Wollstein and Meltzer (9) also give this time as the period of the maximum reaction. The average amount of strophanthin per kilo of body weight injected was 0.125 mg., which was practically the same as the lethal dose required in the normal dogs. The range of the dosage for the infected dogs varied from 0.099 to 0.151 mg. per kilo of body weight. In six of these dogs the amount of strophanthin injected fell within 11 per cent of the average. The remaining four dogs, Nos. 3, 5, 11, and 12, showed respectively a variation of 20 per cent above, 16 per cent below, 20 per cent below, and 15 per cent above. If the four animals showing the greatest variation are omitted from the series it is found that the average m. l. d. of the remaining six dogs is still 0.12 mg. The time which elapsed between the commencement of the injection and the death of the animal varied from 55 to 115 minutes, the average time being 82 minutes. A comparison, then, of the normal and infected dogs shows the lethal dose of strophanthin in each to be the same.

Autopsy Findings.—Autopsies were performed on all the cats and dogs in both the normal and infected series, except on the cats in Group I that survived the injection of strophanthin and on the cats in Group II that survived the infection. Autopsies performed on cats of the control series (Group I) killed by strophanthin showed the lungs to be normal. Two cats of Group II, Nos. 34 and 36, died during the height of the infection. No. 34 showed a massive consolidation of the entire left lung, and also of the middle and posterior lobes of the right. No. 36 showed scattered areas of consolidation throughout all the lobes of both lungs, the left lung being almost entirely consolidated. A well marked fibrinous exudate covered the

pleura and a moderate amount of fluid was found in both pleural cavities. Two other cats belonging to this group died at a period three weeks after infection, but their death was evidently due to an intercurrent disease. Autopsies on these animals showed small, scattered, abnormal areas in both lungs suggestive of an old pneumonic process.

Autopsies were performed on all the animals of Group III. The lungs in each instance showed definite signs of consolidation. The involvement varied in extent from a portion of one lobe to an entire lung. The gross examination showed the area involved to be deeply congested and the overlying pleura to be somewhat dull and lacking in luster. In a number of the animals the pleura showed the presence of a small amount of serofibrinous exudate. Two animals, Nos. 33 and 55, developed a fibrinopurulent pleurisy, and one, No. 33, also showed a well marked pericarditis. The portion of the lung involved was usually firm, inelastic, and friable. On section the lungs were firm; the cut surface was uniformly dark red with a tendency to be dry. On compression, a small quantity of bloody fluid exuded from the surface. Small portions of tissue cut from the consolidated areas sank in water. The gross appearance of the lungs in dogs was similar to that in the cats. Blood cultures were made at the time of autopsy from the heart's blood in fourteen cats; of these, twelve contained pneumococci.

Microscopic examination of the lung tissue showed the alveoli to be filled with an exudate consisting chiefly of leucocytes. A few large lymphocytes, red blood cells, and desquamated epithelial cells were also present. Only small quantities of fibrin were contained in the alveolar exudate. The amount of fibrin found in the pneumonias in cats was slightly more than that found in the dogs. The walls of the alveoli were only moderately congested. The bronchi contained small amounts of exudate, consisting chiefly of red and white blood cells with particles of desquamated epithelium and small amounts of fibrin.

Electrocardiograms.—Electrocardiograms were made of ten infected cats, of seven normal dogs, and of all the infected dogs. Non-polarizable electrodes were placed on the right fore and the left hind leg. Control curves were obtained before infection of the

animals, before injection of strophanthin, and also at ten minute intervals after the injection was begun. The principal changes found to follow the injection were increase in the conduction time, alterations in the size and form of the T wave, and the production of extrasystolic irregularities. Changes in the P-R time occurring during the course of the strophanthin injection were seen in four of the ten cats. In these there was an increase of 0.02 of a second or more. In the remaining six cats there was no alteration in the conduction time. Changes in the P-R time were also seen in five of the normal and in six of the infected dogs. In all of these there was an increase of 0.02 of a second or more (Figs. 1 B and 3 C). The remaining dogs of both groups showed no important change in the P-R time. Blocking of the auricular impulse occurred in two of the normal and in three of the infected dogs (Figs. 2 B and 3 B).

Alterations in the T wave have recently been shown to occur in man during the administration of digitalis (10). The curves of cats and dogs displayed similar changes (Figs. 4, 5, and 6). Of the ten cats, a negative T wave was present in the control curves of two, and became deeper after the injection (Fig. 5 B). In two others a diphasic T wave became negative, and in a fifth an upwardly directed T wave became markedly diminished in height (Fig. 6). In the remaining five cats, the strophanthin produced no demonstrable change in this wave. In two of the seven normal dogs, the T wave in the control electrocardiogram was positive. After injection it became diphasic in one, and negative (Fig. 1 B) in the other. The remaining five dogs all showed negative T waves in the control curve. On the form of these strophanthin appeared to have no influence. In the ten infected dogs changes occurred more often. In six animals the T wave was positive in the control curve and became negative under the influence of strophanthin (Figs. 3 B and 3 C). In the seventh a negative T wave became diphasic. In the remaining three dogs negative T waves in the control curve became deeper under the influence of the drug (Fig. 4 B). Changes in the T wave were observed, therefore, five times in ten cats, and in twelve of seventeen dogs.

Extrasystolic (11) irregularities occurred in all the animals following the injection (Figs. 3 D and 6 B). Several of the cats

which received the average lethal dose of strophanthin and survived showed an irregularity of this type to a marked degree. When electrocardiograms were made of these animals, twenty-four hours later, the heart rhythm was again normal (Fig. 6). In one cat that survived the injection of strophanthin, persistence of a change of shape in the T wave continued for a period of at least forty-eight hours (Fig. 5). In all the dogs and in the cats that died, extrasystoles multiplied in frequency. At the time of death fibrillation of the ventricles occurred.

SUMMARY AND DISCUSSION.

The results of these experiments permit a comparison of the action of strophanthin in the normal and the infected animals. In cats the percentage of deaths following the injection of 0.1 mg. of strophanthin per kilo of body weight was the same in normal and in pneumonic cats. The number of recoveries in this series was larger than was expected. This result was due to the fact that the dose injected was not the lethal dose, but the average lethal dose (0.1 mg.) determined by Hatcher and Brody (7) and by Eggleston (8). The doses which the latter actually injected ranged from 0.085 mg. to 0.16 mg.; from these the average minimal lethal dose was calculated. In adopting the average dose as the standard one to inject, those of our cats that required more than 0.1 mg. naturally survived. The death rate was therefore low. The plan employed in dogs differed from that used in cats. A lethal dose was injected in each dog. Death occurred when an average of 0.12 mg. of strophanthin per kilo of body weight was injected. The same dose was required in normal and pneumonic dogs. The effect of strophanthin in both groups of infected and non-infected cats and dogs is, therefore, identical.

Whether the uniformity of strophanthin action in the two experimental groups may serve as the basis for assuming a like uniformity of action in normal individuals and in pneumonia patients is a subject which requires further analysis. The difficulty in transferring the experimental results to patients lies in the question of whether the type of pneumonia produced in animals is the same as that found

in man. Clinically, the two diseases present both resemblances and differences. The animals become definitely ill and show the symptoms already described. The illness, however, is of short duration and apparently reaches its height in the majority of animals in twenty-four to seventy-two hours. Before the expiration of this time, the temperature frequently returns to normal. Many of the infected animals, when they survive, recover in three to five days. The mortality in dogs infected with pneumococci is given by Lamar and Meltzer as 16 per cent. In the present series of twelve infected cats, the mortality was also 16 per cent. These findings differ from human pneumonia in the following particulars: The infection is not so severe; the temperature, though elevated at first, soon falls; the duration of the disease is short; and convalescence is rapid. The mortality is slightly lower. Musser and Norris (12) give the human mortality at 21.06 per cent. Pathologically the two diseases also show differences. The gross appearance of the lungs is not dissimilar, but in the animals the consolidated portions are somewhat dry and they fail to show a stage of gray hepatization (5). The amount of fibrin present is small. There is comparatively slight congestion of the alveolar walls and of the walls of the bronchi.

The relation of experimental pneumonias to the human disease has been discussed by a number of investigators. Almost all believe that the two types are similar, if not identical. Among the first to express this opinion was Sternberg (13); and later Gamaléia (14), Prudden and Northrup (15), Kinyoun and Rosenau (16), Wadsworth (17), Lamar and Meltzer (5), Wollstein and Meltzer (9) coincided with his view. Lamar and Meltzer, especially, have insisted on the identity of the two processes. On the other hand, Welch (18) in his study of experimental pneumonia, says: "Many inoculations of cultures of virulent pneumococci into the trachea and lungs of dogs have been made in my laboratory by Dr. Canfield and myself, but in no instance were we able to produce an inflammation of the lungs which we were willing to identify with acute lobar pneumonia as found in human beings." But he adds that, in the majority of experiments, there was no demonstrable consolidation and that pleurisy and more or less extensive areas of pneumonia were produced only in a few animals.

The inference consequently cannot be drawn that an effect obtained with strophanthin in the experimental disease may be anticipated in man. The striking similarity in action in infected and uninfected animals renders it likely, however, that the usual action of the drug in man may be expected in the presence of pneumonia. We have accumulated evidence, to be published later, which shows that this action actually takes place in the human disease. As far as evidence obtained electrocardiographically is concerned, our experiments show that strophanthin causes the same electrical changes in the heart when the animals are infected as it does under normal conditions.

CONCLUSIONS.

1. When a like amount of strophanthin is injected intravenously, the mortality is the same in both normal cats and in cats suffering from experimental pneumonia.

2. The minimum lethal dose of strophanthin is the same in normal dogs and in dogs suffering from experimental pneumonia.

3. The presence of an acute infection in these animals does not interfere with the action of strophanthin on the heart.

4. Electrocardiographically the changes occurring in the heart's action when strophanthin is injected are found to be similar in normal and in infected animals.

5. The identity of strophanthin action in infected and normal animals renders it probable that a like similarity may be anticipated in man, under normal conditions and in pneumonia.

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EXPLANATION OF PLATES.

In the following electrocardiograms leads were taken from the right fore to the left hind leg. In all figures the divisions of the abscissæ equal 0.04 of a second, the divisions of the ordinates equal 10^{-4} millivolts.

PLATE 74.

FIG. 1, A and B. These curves were taken from Normal Dog B. In A, made immediately before the injection of strophanthin, the P–R interval is 0.07 to 0.08 of a second. The T waves are prominent and upwardly directed. In B, taken 25 minutes after the injection was begun and when 0.43 mg. of strophanthin had been injected, P–R time increased to 0.11 or 0.12 of a second. The T wave is negative. The time since the commencement of the injection and the amount of strophanthin injected are given except in Fig. 3. In the cat curves the time only is indicated.

FIG. 2, A to C. Curves taken from Normal Dog C. Curve A was taken before the injection of strophanthin. The rhythm is regular. The P–R interval is 0.08 to 0.09 of a second. The T waves are negative. Curve B was made 65

minutes after the injection was begun; strophanthin 0.76 mg. had been injected. Complete dissociation between auricles and ventricles is shown. The rate of the auricles is much reduced. Curve C was made 20 minutes later than B; strophanthin 0.85 mg. had been injected. It shows a succession of ectopic ventricular beats. Two distinct types are seen. In the first and last parts of the curve the impulses arose in the wall of the right ventricle; those between, in the wall of the left ventricle. The latter show considerable variation. Auricular complexes cannot be distinguished.

PLATE 75.

FIG. 3, A to D. These curves were taken from Infected Dog 1. Curve A was made before the injection of strophanthin. The P–R time is 0.10 of a second. The T wave in some of the complexes is flat, in others it is composed of two parts, a low upwardly directed portion and a small negative wave. Curve B was taken 65 minutes after the injection of strophanthin was begun. The P–R time is lengthened to 0.15 of a second. The T waves consist of three parts: first, a gradual upward rise following the S wave; second, a rather sharp downward deflection; and third, an upward rise. Every third auricular impulse is blocked. Curve C was taken 10 minutes after Curve B. The complexes are normal. The P–R time is 0.15 of a second. The ventricular complex is similar to that seen in Fig. 2 B, the principal change being in the T wave. Ventricular extrasystoles occur which are probably of left ventricular origin. The T wave accompanying these is positive. Curve D was taken 25 minutes later than C; strophanthin 1.09 mg. had been injected. No auricular waves appear. In each pair of ectopic ventricular beats the first member has its origin in the left ventricle, and the second in the right ventricle. The third pair forms an exception; here the order is reversed.

FIG. 4, A and B. These curves were taken from Infected Dog 11. Curve A was made before the injection of strophanthin. The P–R time is 0.08 of a second. The T wave is made up of three parts, a small positive wave following directly on the S wave, a small negative, and a small positive wave. Curve B was made 15 minutes after the injection of strophanthin was begun; 0.24 mg. had been injected. The P–R time is unchanged. The T wave is composed of two parts, a negative portion of considerable depth and a small upward part.

PLATE 76.

FIG. 5, A to D. These curves were taken from Infected Cat 63. The animal survived the injection of strophanthin. Curve A was made before the injection. In the earlier portion of the curve the P wave has a normal relation to the R wave; later it gradually merges into the R wave until it disappears. The excursion of R is now increased and S is decreased. The T wave consists of two portions, a flat or upwardly directed portion and a small negative wave. Curve B was taken 13 minutes after the injection of strophanthin was begun. The P–R time is 0.07 of a second. The T wave consists of two portions, a flat or upwardly directed portion and a negative part of much greater extent than in Curve A. Curves C and D were taken 24 and 48 hours after the injection of strophanthin, respectively. In both curves the P wave is absent and in this

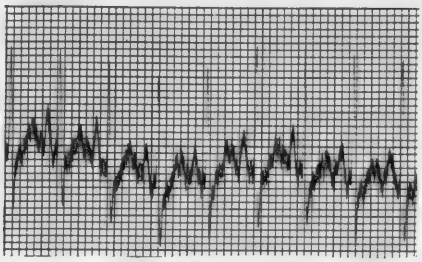


Fig 1.A. Dog B.

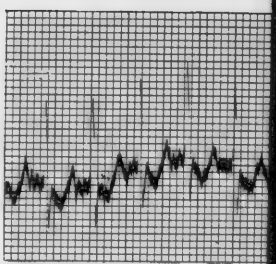


Fig.1B. 25 min. 0.

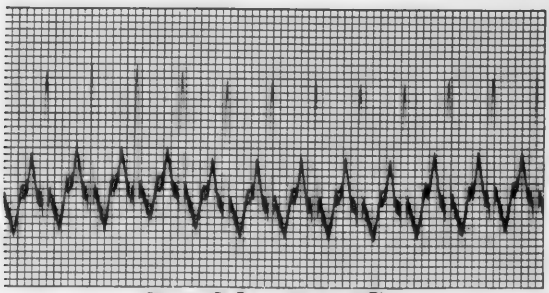


Fig.2A. Dog C.

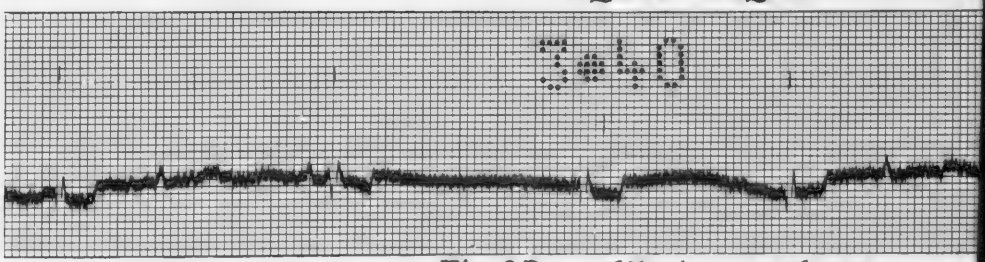


Fig.2B. 65 min. 0.76 mgm.

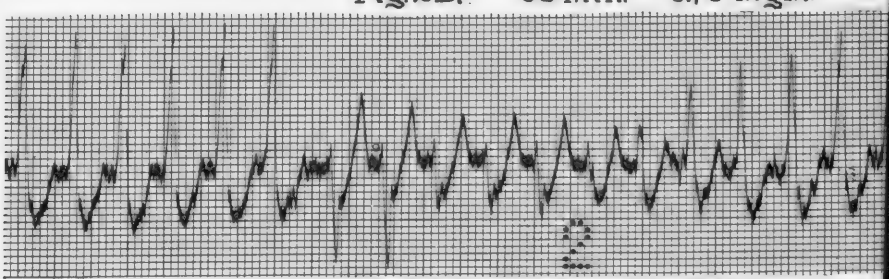


Fig.2 C. 85 min. 0.85 mgm.

(Jamieson: A

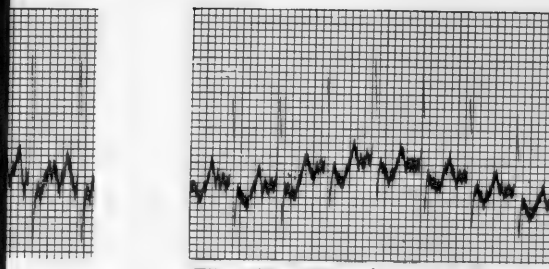


Fig.1B. 25 min. 0.43 mg

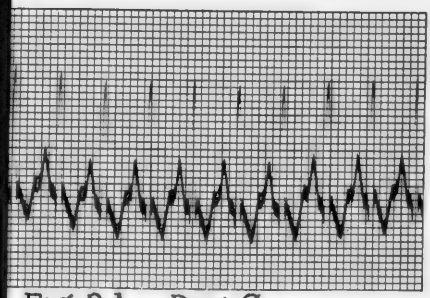
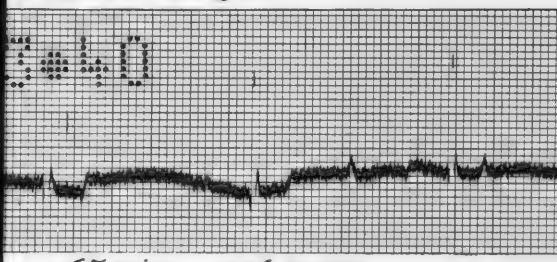
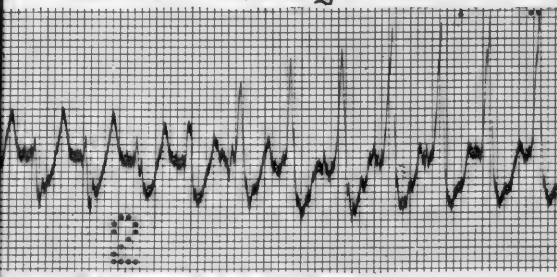


Fig.2A. Dog C.



65 min. 0.76 mgm.



85 min. 0.85 mgm.

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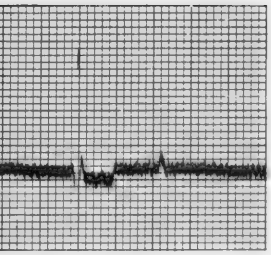


Dose of Strophanthin in Animals.)

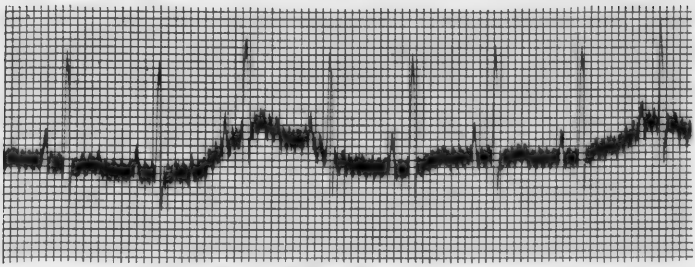
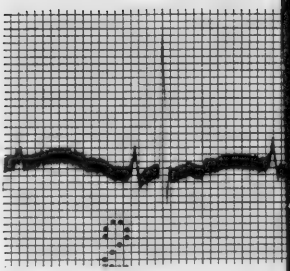


Fig. 3A. Dog 1.



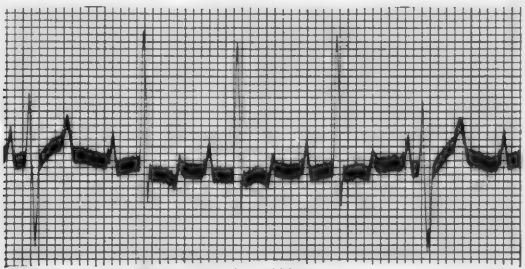
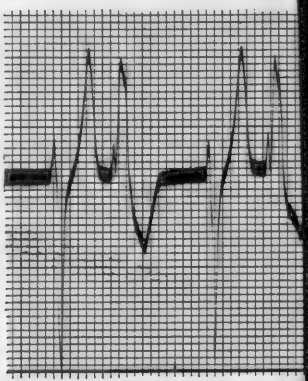


Fig. 3C. 75 min.



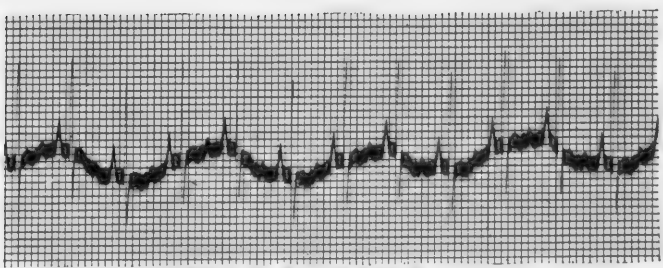
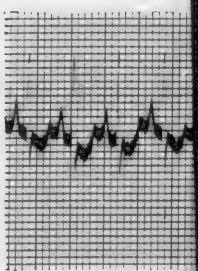


Fig. 4A. Dog 2.



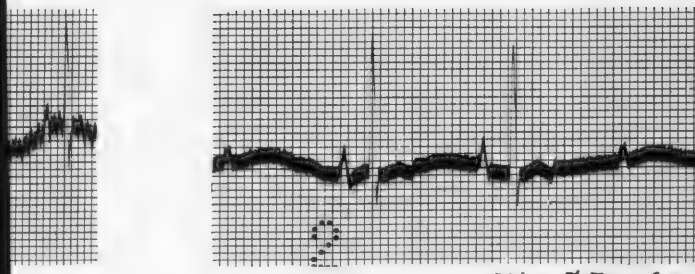


Fig. 3B. 65

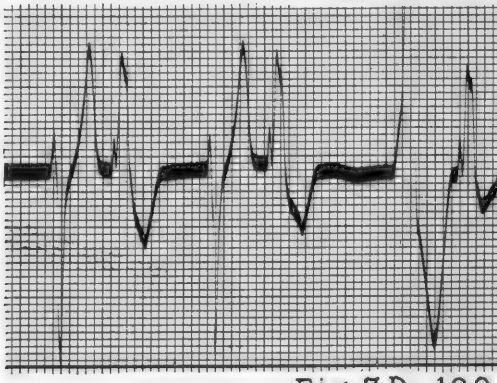


Fig. 3D. 100

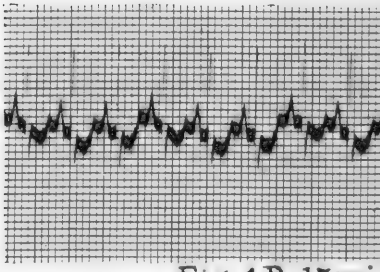


Fig 4B. 15mi

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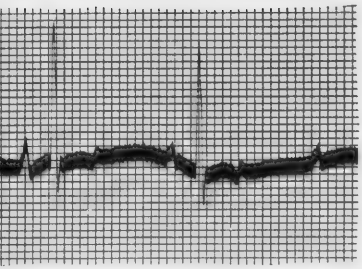

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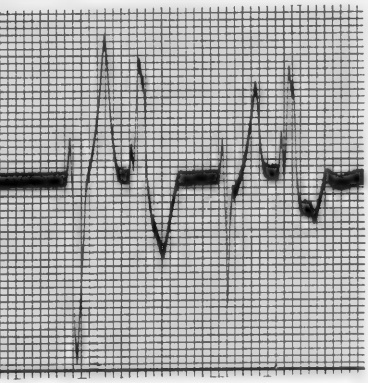

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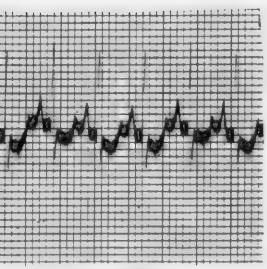

n. 0.24 mgm.

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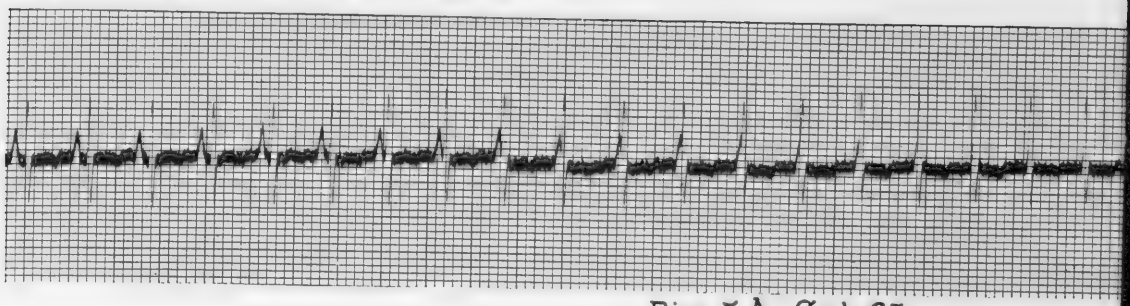


Fig. 5A. Cat 63.

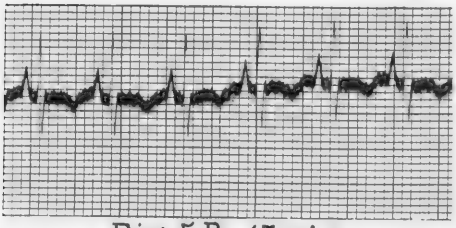


Fig. 5B. 13min.

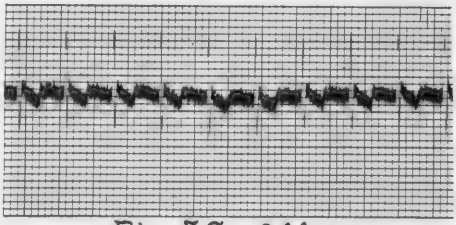


Fig. 5C. 24hrs.

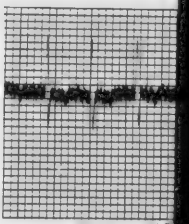


Fig. 5I

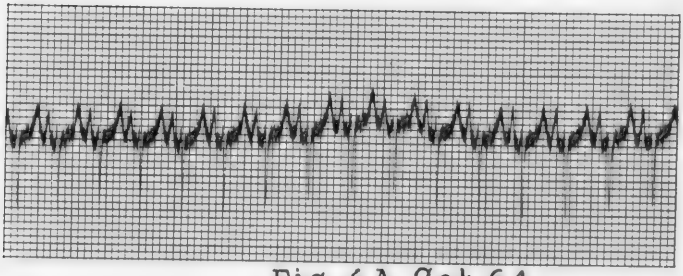


Fig. 6A. Cat 64.

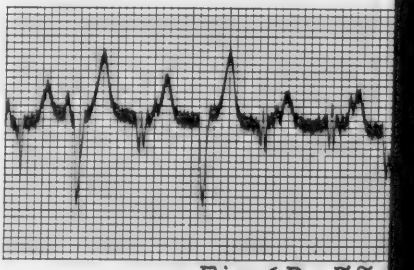


Fig. 6B. 33r

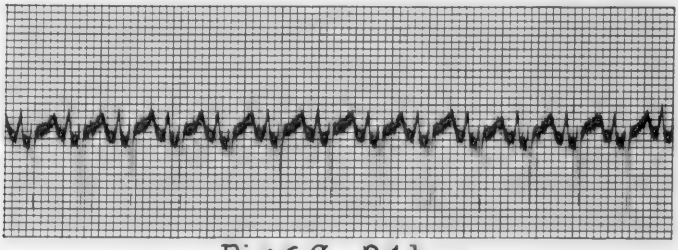


Fig. 6C. 24hrs.

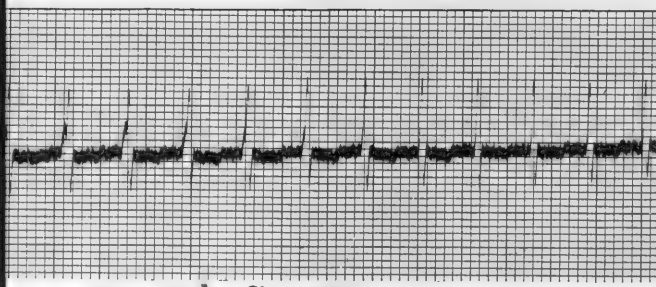


Fig. 5A. Cat 63.

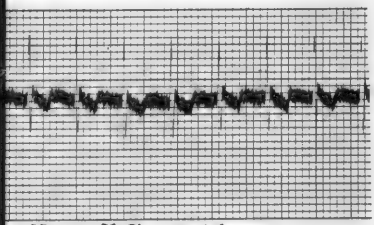


Fig. 5C. 24 hrs.

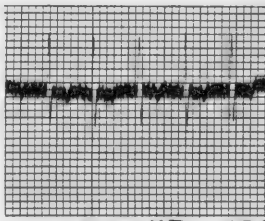
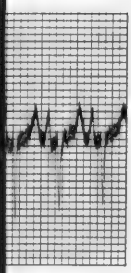


Fig. 5D. 48



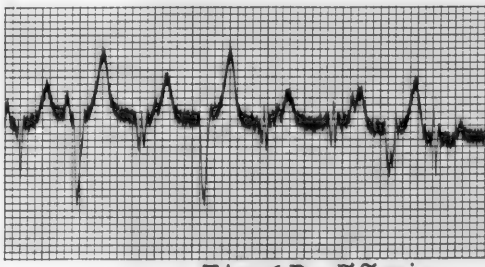


Fig. 6B. 33 min.

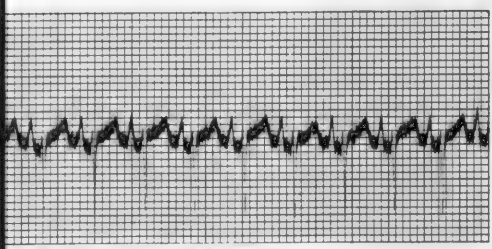


Fig. 6 C. 24 hrs.

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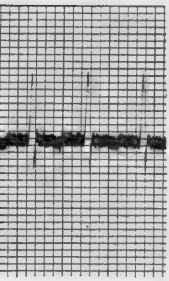
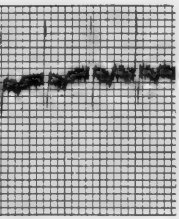
PLATE 76.





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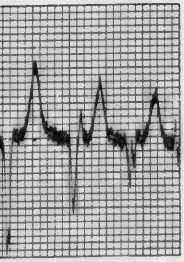

trophanthin in Animals.]

respect they resemble the last portion of Curve A. A negative T wave has persisted in C and D, though in the latter T wave is more like the form seen in Curve A.

FIG. 6, A to C. These curves were taken from Infected Cat 64. This animal survived the injection of strophanthin. Curve A was made before the injection. The heart rhythm is normal. The P–R time is 0.07 of a second. The T wave is positive. Curve B was taken 33 minutes after the injection of strophanthin was begun. It shows a completely irregular heart action. No P waves are discernible. The ventricular complexes are ectopic, have various forms, and have their origin at several points in the walls of the right and left ventricles. Curve C was taken 24 hours later and shows the return of the heart to a normal rhythm. The P–R time is the same as in Curve A. The T waves are positive.

FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE.

I. Tumor Incidence and Tumor Age in Various Strains of Mice.

By A. E. C. LATHROP and LEO LOEB, M.D.

(*From the Department of Pathology of the Barnard Free Skin and Cancer Hospital, St. Louis.*)

(Received for publication, June 2, 1915.)

The starting point for our investigations was the observation of the endemic occurrence of tumors in animals.[1]

At that time, as well as on later occasions, we emphasized the fact that hereditary factors might be the cause of this endemic occurrence and that we had not been able to find any direct indication of infection; and we had furthermore occasion in a later publication to point out the importance of analyzing these conditions through breeding experiments. In 1907 we published some observations made at the mouse farm of Miss Lathrop, in Granby, Mass., which rendered it probable that the frequency of tumors in mice at certain places was in all probability due, not to infection, but to hereditary transmission in certain families.[2] Keeping mice in cages, where formerly tumor mice had lived, did not increase the ratio of cancer among those mice, while there was some indication that mice belonging to different families and kept on the same farm in Granby, Mass., showed a different tumor incidence, although the living conditions were approximately the same for the various families.

In 1910 we were enabled to resume these investigations on a larger scale on the same mouse farm in Granby, and in the following year we published the tree of one of the families of mice under our observation in which the hereditary transmission of tumors had been apparent.[3] Since our publication in 1907 there

[1] Loeb, L., On Carcinoma in Cattle, *Medicine*, 1900, vi, 286. Ueber das endemische Vorkommen des Krebses beim Tiere, *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1904, xxxvii, 235.

[2] Loeb, Further Observations on the Endemic Occurrence of Carcinoma, and on the Inoculability of Tumors, *Univ. Penn. Med. Bull.*, 1907–08, xx, 2.

[3] Loeb, Ueber einen Kontakt-Kombinationstumor bei einer weissen Maus, *Centralbl. f. allg. Path.*, 1911, xxii, 993.

appeared two investigations, one by Tyzzer[4] and a more recent one by Murray,[5] dealing with the heredity of tumors in mice. Both compared the frequency of tumors in a certain number of mice which were directly descended from tumor mice with those which had no tumor mice in their immediate ancestry. Tyzzer considers that his results suggest a possible influence of heredity, and Murray also considers his conclusions not yet as quite definite. Inasmuch as the results of Murray are more recent and are based on more extensive observations than those of Tyzzer, it might be of interest to analyze briefly the facts on which Murray's conclusions are based. Murray states that all the mice concerned in the experiments are descended ultimately from animals known to have suffered from cancer. "When new spontaneous cases of cancer in mice bred outside the laboratory are obtained, males bred in the laboratory from cancerous stock are mated with them, and as time goes on the process is repeated, so that a large number of animals with a composite known ancestry is obtained. This continual introduction of fresh cancerous stock to the parentage was decided on to eliminate, as far as possible, the influence of other indeterminate peculiarities which might conceivably influence the incidence of cancer indirectly in the nearly related animals derived by inbreeding from a single pair." Murray based his conclusions on the observation of 562 females which attained the age of 6 months or more. These 562 mice were divided into two unequal groups, one in which cancer occurred either in the mother, one or the other grandmother, or in all three, and another in which cancer was more remote in the ancestry. In the first lot of mice 18.2 per cent, in the latter 8.2 per cent tumor mice were found.

The principal criticism which can be made against conclusions based on this procedure is the following: Mice whose mothers or grandmothers had no tumors might belong to strains rich in tumors. In this case their offspring would in all likelihood develop many tumors, while mice whose direct ancestry had tumors might belong to strains very poor in tumors and their offspring would then develop less tumors than the offspring of non-tumor mice. For instance, if we should select non-cancerous female mice from our strains "English," "8½ plus English," "Michigan Wild plus 101," "Nov. 3d Strain," "Nov. 8th Strain," and compare the cancer rate of their offspring with the cancer rate of tumor mice from the strains "8½," "London," "Heitler," "Silver," "Cream," "German plus Carter," we would find that the offspring of the non-tumorous females is much richer in tumors than the offspring of tumorous animals. There is therefore a very large factor of chance in the results obtained by Murray. Accidentally the mice whose grandparents or parents had had tumors belonged to strains somewhat richer in tumors than the strains to which the other mice belonged. Definite conclusions can only be obtained if we use strains the tumor rate of which is known. These results we published in 1913.[6] We showed

[4] Tyzzer, E. E., A Series of 20 Spontaneous Tumors in Mice, with the Accompanying Pathological Changes and the Results of the Inoculation of Certain of These Tumors into Normal Mice, *Jour. Med. Research*, 1907–08, xvii, 155.

[5] Murray, J. A., Cancerous Ancestry and the Incidence of Cancer in Mice, *Fourth Scientific Report of the Imperial Cancer Research Fund*, 1911, 114.

[6] Lathrop, A. E. C., and Loeb, L., The Incidence of Cancer in Various Strains of Mice, *Proc. Soc. Exper. Biol. and Med.*, 1913, xi, 34. Loeb, L., Some Recent Results of Cancer Investigations, *Lancet-Clinic*, 1913, cx, 664.

among other facts on the basis of very extensive observations that different strains kept at the same farm differed markedly in their tumor rate, that this difference in tumor rate was a characteristic which was maintained approximately constant through several generations, and we gave definite figures for the frequency of tumors in different strains. Thus the great importance of the factor of heredity in the transmission of tumors in mice was definitely proven.

Since 1913 we have continued our investigations and we wish to report (1) the extension of the work, (2) more detailed figures concerning the cancer rate and cancer age in the successive generations of the various strains, and (3) some new facts especially regarding the relation between cancer rate and cancer age. These data will also form a basis for succeeding communications.

We found it best to divide the mice into three classes, according to age: (1) in Class I were mice 7, 8, 9, 10, 11, and 12 months old; (2) in Class II were mice 13, 14, 15, 16, and 17 months old; (3) in Class III were mice 18 months old and older. Mice dead without tumor below the age of seven months are disregarded. In our tables we give the generations of the mice as F_1, F_2, F_3, and so on, designating as F_1 the first generation which we included in our new records. On the left side of the table we state the number of mice that die without tumors, on the right side those with tumors. First we give the total figure of observed mice, then in brackets the number of mice that die in each of the three periods of life. The Roman figures after each Arabic figure indicate the period of age during which a certain number of mice died. We give first the absolute figures and below in each case the percentage figures. In the summary of the various strains we add the percentage of mice dying with and without tumors in the respective periods expressed in per cent of the total number of mice dying in this period. We considered only female mice and included all the subcutaneous tumors. The large majority of these are the common adenocarcinomata and carcinomata. There occur a few other tumors, as, for instance, squamous cell carcinoma and sarcoma. The number of these latter tumors is, however, in our material apparently so small that the results cannot be affected by including all the subcutaneous tumors in our calculations. Tumors of internal organs were disregarded at present. We shall report on this phase of our work on a

later occasion after the microscopic study has been concluded. Macroscopically visible tumors in internal organs of mice are of little significance for our present studies. While the material on which our conclusions are based is great, we intend to continue our work, and if there should be an error in our conclusions, it will in all probability be cleared up by our further experiments.

I. Tumor Incidence and Tumor Age of Non-Hybrid Strains.

In this class we consider strains, which, though of composite origin, were kept inbred in our experiments for a number of generations.

1. Strain English.

These are mice imported from England in 1905. Their offspring were brought to Granby, Mass., in the spring of 1906. The mice have a dull reddish color and their offspring have various colors, especially sable, agouti, and dull reddish. Later another female mouse of the same English strain with a bright red color is added. By breeding various red colored mice together an attempt is made to obtain red stock. However, among the offspring there are many black, sable, agouti, and chocolate colored animals, but rarely a mouse with a good red color. All the mice belonging to this strain were derived from a very small number of animals. The strain had been propagated for a number of years at the time when we started our experiments. The same holds good in the case of the majority of other strains. It may have been different in those strains which had been more recently imported, as the "European" and "German," "Heitler" and "London." But even in the Heitler and London the fact that the results even in small groups are, on the whole, concordant with each other does not make it probable that the various individuals within a strain which we used for propagation represented very different kinds of mice. Selection among the original gave, after breeding through several generations, a strain with a good proportion of black-eyed tans, and another with a small proportion of good black-eyed reds. There were also occasionally pink-eyed mice in these groups. One pink-eyed reddish tan colored male of English origin was mated to white females of Massachusetts origin. The offspring of this group again showed various colors, some of them white. These whites bred together for one generation gave again pure white offspring. The other offspring of the reddish tan male and white Massachusetts females were mostly black and sable in color. They all had black eyes. The latter when bred together without selection had generally dull colored offspring, but there were a very small number with pink eyes and a reddish or tan color, and two or three of a dull bluish smoke shade. These various pink-eyed animals had all pink-eyed offspring, usually of a non-characteristic color in the first generation, but in the succeeding generations the clear tan shade appeared; these tan mice, when bred together, bred true. Their offspring were the only mice which had exactly this color. These tan colored

mice gave origin to the English substrains "101" and "Tan." The latter are mostly the offspring of Tumor Mice 121 and 122. They had therefore all tan color. Other mice of the same origin had, as mentioned above, a dull bluish color; there were two or three of those among the grandchildren of the original pink-eyed reddish tan male and the white Massachusetts female. These bluish mice bred together had some offspring of the same color and a few with distinct silver color. The silver colored were rare at first. Mice with somewhat similar, approximately silver color occurred also occasionally among the original English mice, which had no admixture of white Massachusetts stock. Through several generations these silver mice were added to the silver mice of the mixed origin. These silver mice selected and interbred gradually bred true. Occasionally, however, there occurred among them some mice of lighter, almost silver fawn color. The latter bred together also breed true and have only silver fawn offspring. Thus we have four distinct substrains of the English strains, which are characterized by their color which remains distinct: (1) the "Tan" mice with pink eyes, mainly offspring of Tumor Mice 121 and 122; (2) the substrain "101," also tan mice; (3) the "Silver"; and (4) the "Silver Fawn."

In addition to those we have two substrains in which mice of various colors occur: (1) "English Sable," members of the original English family. One pair of sable color was selected and gave origin to this substrain. This substrain has never had mice with the red and tan colors which some of the original English mice showed, but had many sable, some black, and many white mice. As white males were usually kept, the colored mice have now become eliminated and all "Sable" are at the present time white. The colored mice belonging to this substrain have never had pink eyes, but the white mice at present are pink-eyed, like ordinary white mice. (2) The last substrain is "English A," non-selected groups from the general stock. There were some groups all sable in color, some all red or orange; in other groups various colors were represented, sable, agouti, black, white, dull tans, and dull reds. In no case, however, did a real Silver occur in such a mixed group. They occurred only a few times in earlier generations and were either discarded if of dull color, or added to the first two Silver generations, if of fairly clear color. Mice in this strain differed not only in their color, but also in their resistance. On the whole, the red and yellow pink-eyed varieties of English mice are not very rugged and not satisfactory as breeders. The females of the 101 strain were prolific, they very frequently had new litters, but they were usually small and died often at early ages. All the Silver as well as the 101 and Tan had pink eyes. None among the English Sable, with the exception of the white ones, had pink eyes. Of the substrain A some had pink eyes, but the majority were black-eyed. No. 481 a and her descendants had pink eyes, although they are mice of a relatively dark, dull agouti color.

The following substrains were thus distinguished:

1. English A, composed of various groups of English, not line bred.
2. Family 101, the offspring of pink-eyed tans.
3. English Tan E, the offspring of Tumor Mice 121 and 122.
4. English Sable.

5. English Silver.
6. English Silver Fawn.

It was of special interest to compare the tumor rates in the various groups which had a similar origin and to determine whether the various groups differed markedly in their tumor rates.

(a) Earlier groups.

1. Substrain English A.

Without tumors.	With tumors.
44 (33 I 4 II 7 III)	71 (25 I 39 II 7 III)
37% (75% I 9% II 16% III)	63% (35% I 55% II 10% III)

(b) Later groups.

12 (7 I 5 II)	21 (13 I 7 II 1 III)
36% (58% I 42% II)	64% (62% I 33% II 5% III)

(c) Additional records (winter, 1914–15).

8 (3 I 4 II 1 III)	19 (14 I 5 II)
30% (38% I 50% II 12% III)	70% (79% I 21% II)

We see that the tumor rate in the same group at different periods remained approximately the same (63, 64, and 70 per cent), that a large per cent of the tumors appear in the first period of life, and a large part of the remaining tumors in the second period. As a result of the high tumor incidence in young mice, the mortality is in early life so great that the number of mice attaining an old age is relatively small.

The figures for the total of this lot are:

Without tumors.	With tumors.
64 (43 I 13 II 8 III)	111 (52 I 51 II 8 III)
37% (67½% I 20% II 12½% III)	63% (47% I 46% II 7% III)
II + III 26% III 50% I + II 35% I 35%	II + III 74% III 50% I + II 65% I 65%

In two cases the offspring of tumor mice belonging to this group were followed separately. Through four generations the offspring of Tumor Mouse 481 a, (dull agouti with pink eyes) were recorded.

Without tumors.	With tumors.
3 (3 I)	11 (8 I 3 II)
21% (100% I)	79% (72% I 28% II)

In this case the result is in accordance with the behavior of the group as a whole.

In the offspring of Tumor Mouse 281, which were followed through five generations, the tumor rate is very much lower.

(a) Earlier generation.

Without tumors.	With tumors.
7 (7 I)	1 (1 II)

(b) Later generations.

5 (1 I 3 II 1 III)	2 (1 I 1 II)
Total: 12 (8 I 3 II 1 III)	3 (1 I 2 II)
80% (68% I 24% II 8% III)	20% (34% I 66% II)

It is possible that we accidentally isolated in this last family recessives with a low tumor rate. But as the mice died early in this case, and the number of animals we observed is small, we must leave this question at present undecided.

2. Substrain Family 101.

No. 101 was a pink-eyed English Tan tumor mouse from a family in which all three females had tumors.

	Without tumors.	With tumors.
F_1	3 (3 I)	
F_2	6 (6 I)	10 (9 I 1 II)
	37% (100% I)	63% (90% I 10% II)
F_3	7 (3 I 4 II)	22 (17 I 5 II)
	24% (43% I 57% II)	76% (77% I 23% II)
F_4	9 (5 I 4 II)	27 (17 I 9 II 1 III)
	25% (55% I 45% II)	75% (63% I 33% II 4% III)
F_5	2 (1 I 1 II)	6 (1 I 5 II)
	25% (50% I 50% II)	75% (17% I 83% II)
F_6	2 (2 I)	5 (2 I 3 II)
	28% (100% I)	72% (40% I 60% II)
Total:	29 (20 I 9 II)	70 (46 I 23 II 1 III)
	29% (69% I 31% II)	71% (66% I 33% II 1% III)
	II + III 28% I 30% I + II 29%	II + III 72% I 70% I + II 71%

We see that we have here to deal with a high tumor rate (71 per cent), that the majority of the tumors appear in the first period of life, and the rest in the second period, and furthermore that in the six generations which we observed in this family the tumor rate and

the tumor age were about the same. If we disregard the mice dying in the first period of life, the tumor incidence remains about the same (72 per cent). In this family a tumor was observed to appear in a six months old mouse.

3. Substrain English Tan E.

Offspring (pink-eyed) of two early Tan Tumor Mice 121 and 122.

Without tumors.	With tumors.
3 (3 I)	8 (6 I 2 II)
27% (100% I)	73% (75% I 25% II)

This is a small group of similar character to the preceding family. Here tumor incidence and tumor age are similar to those in 101.

4. Substrain English Sable.

(a) Older records.

	Without tumors.	With tumors.
F_1	3 (2 or 3 I 1 uncertain II)	6 (3 I 3 II)
F_2	12 (11 I (6 of these below 6 months) 1 III)	40 (33 I 5 II 2 III)
F_3	11 (8 I 1 II 2 III)	25 (21 I 3 II 1 III)
F_4	3 (1 I 2 II)	21 (17 I 3 II 1 III)
F_5	0	5 (4 I 1 II)
Total:	29 (22 I 4 II 3 III)	97 (78 I 15 II 4 III)
	23% (76% I 14% II 10% III)	77% (81% I 15% II 4% III)
	II + III 29%	II + III 71%

(b) Newer records.

F_2	2 (1 I 1 II)	5 (3 I 2 II)
F_3	19 (11 I 7 II 1 III)	24 (14 I 8 II 2 III)
F_4	4 (3 I 1 II)	4 (1 I 3 II)
Offspring of Tumor Mouse 217 1	(1 I)	6 (2 I 4 II)
Total:	26 (16 I 9 II 1 III)	39 (20 I 17 II 2 III)
	40% (62% I 34% II 4% III)	60% (51% I 44% II 5% III)
	II + III 34%	II + III 60%

Total of (a) and (b):

55 (38 I 13 II 4 III)	136 (98 I 32 II 6 III)
28% (69% I 24% II 7% III)	72% (73% I 23% II 4% III)
II + III 31%	II + III 69%

(c) Family of Tumor Mouse 437. (No. 437 raised young while she had a tumor.)

F_1	1 (1 I)	7 (1 I 6 II)
F_2	2 (2 I)	4 (2 I 2 II)
F_3	2 (2 I)	5 (4 I 1 II)
F_4	0	
Total:	5 (5 I)	16 (7 I 9 II)
	24% (100% I)	76% (44% I 56% II)

(d) Additional records (winter, 1914–15).

F_3 9 (4 I 3 II 2 III)	15 (5 I 4 II 6 III)
F_5 7 (2 I 3 II 2 III)	8 (4 I 3 II 1 III)
F_6 0	1 (1 I)
Total: 16 (6 I 6 II 4 III)	24 (10 I 7 II 7 III)
40% (37½% I 37½% II 25% III)	60% (42% I 29% II 29% III)
Total of English Sable:	176 (115 I 48 II 13 III)
76 (49 I 19 II 8 III)	70% (66% I 27% II 7% III)
30% (65% I 25% II 10% III)	II + III 69% III 62% I 70% I + II 71%
II + III 31% III 38% I 30% I + II 29%	

If we compare the total of English Sable with the total of English 101, we see that the figures are very similar as to the incidence of cancer and the age at which cancer appears. In both cases (without consideration of the mice alive in the various periods) the majority of tumors appear in the first period of life. In English A the tumor incidence is only slightly less, and here also the first period of life is the one in which there appear more tumors than in any other period; but here again the preponderance of the first period over the other periods is not so great as in the case of the Sable and 101. English Tan and English Sable are similar. Within the Sable the various generations and families of individual tumor mice behave in a similar way as the total. Of course, certain variations occur, but they are relatively slight and they are especially noticeable where the number of observed mice is relatively small; only in one family a different tumor rate may prevail, but even here it is not certain. The offspring of mice born while the mother had a tumor are not more liable to have tumors than other mice of the same group (family of Tumor Mouse 437). On the whole, the variations in the incidence of tumors and the age at which they appear follow a parallel course; if the incidence is somewhat lower in a group, the tumors also appear at a somewhat later period of life. At least this parallelism is noticeable in the records within the groups of the English strain just mentioned. It remains to be seen whether or not this parallelism is accidental.

5. Substrain English Silver.

They were bred for color. All the English Silver mice are poor breeders.

(a) Older records.

Without tumors.	With tumors.
74 (29 I 39 II 6 III)	5 (1 I 4 II)
94% (39% I 53% II 8% III)	6% (20% I 80% II)
II + III 92%	II + III 8%

There were 9 groups. In 7 no tumors were observed, in 2 groups tumors appeared. In 1 of these 2 groups 2 out of 21 mice, in the other 3 out of 13, had tumors.

(b) Additional records (winter, 1914–15).

35 (15 I 10 II 10 III)	3 (3 I)
92% (43% I 28½% II 28½% III)	8% (100% I)

Here tumors appeared in 2 groups out of 6. In one there were 2 tumor mice in a group of 10, in the other 1 out of 7 had a tumor.

Total of all English Silver:

109 (44 I 49 II 16 III)	8 (4 I 4 II)
93% (41% I 45% II 14% III)	7% (50% I 50% II)

We see that tumor incidence is the same in both sets (a) and (b). The difference between the other English and the English Silver is most striking. In the latter the tumor rate is very low throughout. Furthermore, it is of interest that out of 15 groups tumors appeared in only 4, and in 3 out of these 4 groups there were more than 1 tumor mouse, while in 11 groups no tumor appeared.

Of great interest is the fact that while the tumor incidence in the English Silver is very low, the mice that had tumors had them at about as early an age as the strains with high tumor rate. The English Silver that did not have tumors in the first or second period of life were not more liable to acquire them in the third period than they were in the first and second periods. It seems therefore that incidence of tumor and tumor age are unit factors which are to some extent independent and transmitted independently of each other. Furthermore, we see here established the interesting fact that there is a linking in this case between the color of the mice and the rate of tumor incidence. A low rate goes with a silver color, while a high rate goes with the sable, tan, and white among the English strains.

6. Substrain English Silver Fawn.

The Silver Fawn have been more prolific than the Silver, but just as delicate. They rarely raise their young. Two groups were kept: (1) 1 to 9 months old, 2 to 11 months old, 1 to 13 months old, 1 to 15 months old, without tumors; (2) their offspring, 1 to 9 months old, 2 to 12 months old, 3 to 13 months old, 2 to 16 months old, without tumors. This record makes it probable that tumors are here as rare as among the Silver. We have therefore split off from the English strain a substrain Silver, in which there went hand in hand with the color a a very low tumor incidence. And from the Silver again another variety of color, Silver Fawn, was split off, in which again there was linked with the color a low tumor incidence.

2. Strain "Cream."

The mice that gave origin to this strain were obtained in the year 1903 from two different sources, Atlantic City and Springfield, Ohio. Up to the year 1911 only the cream colored mice were kept and used for breeding, while the black and sable colored mice which appeared more frequently were discarded. From 1911 on, females of all colors were kept. The females in this strain are poor breeders; the mice grow slowly, but ultimately reach the same size as the other mice. Many of these mice reach old age.

(a) Older records.

Without tumors.	With tumors.
127 (35 I 50 II 42 III)	3 (1 II 2 III)
98% (27% I 39% II 34% III)	2% (33% II 66% III)
II + III 97% III 97.6%	II + III 3% III 2.4%

There were 26 groups in this lot. In one group there appeared 2 tumor mice, and in another group 1 tumor mouse. In 24 groups there appeared no tumor mouse. As in the case of the English mice, the rate of tumor incidence is not essentially changed, if only mice in Periods II and III or mice in Period III of life are considered.

(b) Additional records (winter, 1914–15).

94 (28 I 41 II 25 III)	2 (1 II 1 III)
98% (29% I 44% II 27% III)	2% (50% II 50% III)

These 2 tumor mice appeared in 2 different groups. The tumor incidence is here the same as in Group (a). The low tumor rate in this strain is therefore a constant condition.

Total Cream strain:	
221 (63 I 91 II 67 III)	5 (2 II 3 III)
98% (29% I 41% II 30% III)	2% (40% II 60% III)
II + III 97% III 96%	II + III 3% III 4%

The tumor rate is extremely low, 2 per cent. If we leave the mice dying in the first or first and second period of life out of consideration, the tumor rate is not essentially changed.

Cream X.

Seven mice of the Cream strain (with black, cream, and sable color) were separated from the others, and four generations of these mice were bred. They were called Cream X.

(a) Older records of Cream X.

Without tumors.	With tumors.
44 (20 I 13 II 11 III)	0
100% (45% I 30% II 25% III)	0

(b) Recent records.

86 (28 I 32 II 26 III)	5 (2 I 3 II)
94½% (33% I 37% II 30% III)	5½% (40% I 60% II)
Total of Cream X:	
130 (48 I 45 II 37 III)	5 (2 I 3 II)
96% (37% I 35% II 28% III)	4% (40% I 60% II)

Thus the tumor rate in the Cream X is of the same order as in the Cream, although they had been kept entirely separate. We may therefore conclude that in this case we have to deal with relatively pure strains as far as the tumor incidence is concerned. Of 14 groups of Cream X, 12 were free from tumors; in each of 2 groups (holding 9 and 7 mice respectively) 2 tumor mice were found. If we arrange the Cream X according to generations, we find the following:

F_1	34 (13 I 12 II 9 III)	
	100% (38% I 35% II 27% III)	
F_2	39 (15 I 12 II 12 III)	
	100% (38% I 31% II 31% III)	
F_3	39 (14 I 12 II 13 III)	5 (1 I 3 II)
	89% (36% I 31% II 33% III)	11% (20% I 80% III)
F_4	18 (6 I 9 II 3 III)	1 (1 I)
	95% (33% I 50% II 17% III)	5% (100% I)

Taking Cream and Cream X together we find that tumors occur here apparently later than in the English strain, also later than in the Silver; absolutely the majority of tumors appear here in the second and not in the third period of life.

3. Strain No. 8.

No. 8 is of mixed origin. They are in part descended from mice received from Springfield, Mass., and from Ohio; in part from a pair of plum-silver mice sometime imported from England, but not related to the English strain mentioned previously. From this mixed origin descended some plum-silver females which were in Dec., 1907, mated with the son of a tumor mouse found in Granby. Their descendants are the No. 8 strain. The male, the son of a tumor mouse, was later, when he had grown old, left to his daughters and gave origin to the strain designated 8½. No. 8 is a strain which breeds well and is healthy; the mice live longer than the English strain.

(a) Older records of No. 8.

	Without tumors.	With tumors.
F_2	1 (1 III)	4 (4 III)
F_3	14 (2 I 3 II 9 unknown age)	8 (3 I 2 II 3 III)
F_4	24 (22 I 1 II 1 III)	11 (3 I 6 II 2 III)
F_5	18 (9 I 9 III)	9 (4 I 2 II 3 III)
F_6	39 (8 I 15 II 16 III)	17 (7 I 8 II 2 III)
F_7	29 (19 I 8 II 2 III)	3 (1 II 2 III)
F_9	12 (7 I 3 II 2 III)	4 (1 II 3 III)
F_{10}	12 (3 I 3 II 6 III)	8 (4 II 4 III)
Total:		
	149 (70 I 33 II 37 III 9 unknown age)	64 (17 I 24 II 23 III)
	70% (47% I 22% II 31% III)	
	II + III 60% III 62%	30% (26% I 37% II 37% III)
		II + III 40% III 38%

In this group is maintained through the various generations a medium tumor rate, considerably lower than in the English strain, and the tumor age is higher; also higher than in the English Silver, which have a considerably lower tumor rate than the No. 8 strain.

(b) One tumor mouse, No. 100, from the fourth generation of the No. 8 strain was mated with a male of No. 8, F_4 and had the following offspring:

F_1	0	3 (2 II 1 III)
F_2	4 (1 I 3 III)	6 (1 I 4 II 1 III)
F_3	5 (5 I)	0
F_4	3 (2 I 1 II)	6 (6 I)
Total:		
	12 (8 I 1 II 3 III)	15 (7 I 6 II 2 III)
	44% (67% I 8% II 25% III)	56% (74% I 40% II 13% III)

The tumor rate is here apparently higher than in the No. 8 strain, and the tumor age somewhat lower.

(c) Additional records (winter, 1914–15).

F_8	26 (7 I 13 II 6 III)	2 (2 III)

In the 8th generation the tumor rate is lower than usual. The two tumors appeared here in the last period of life. We have in this case to deal with a relatively small number of mice. Until further observations indicate a constancy in the general lowering of the tumor rate in this strain, we cannot attach too much importance to this observation.

Total of No. 8:	
187 (85 I 47 II 46 III 9 unknown age)	81 (24 I 30 II 27 III)
70% (45% I 24% II 24% III)	30% (29% I 37% II 34% III)
II + III 62% III 63%	II + III 38% III 37%

In this case the tumor rate increases somewhat if we consider only the mice in the second and third periods of life.

4. Strain 8 1/2.

This is a strain closely related to Strain 8. The male (son of a tumor mouse) which gave origin to No. 8 was mated to his daughters, which latter belonged to the No. 8 strain. This strain is less rich in tumors than No. 8, and the tumors appear also at a rather late period of life. This fact may be explained by assuming that the male in this case belonged to a strain less rich in tumors than the females.

Without tumors.	With tumors.
$F_1 + F_2$ 24 (10 I 14 II)	1 (1 II)
F_3 14 (6 II 8 III)	4 (2 I 1 II 1 III)
F_4 51 (14 I 18 II 19 III)	1 (1 II)
F_5 40 (17 I 14 II 9 III)	6 (6 II)
F_6 2 (2 I)	0
Total:	
131 (43 I 52 II 36 III)	27 (6 I 17 II 4 III)
83% (33% I 40% II 27% III)	17% (22% I 63% II 15% III)
II + III 81% III 90%	II + III 19% III 10%

Leaving out of consideration mice which did not reach the second or third period of life modifies the figures only slightly without essentially changing the results.

5. Strain Carter.

This strain is the offspring of a son of a pink-eyed English tumor mouse of orange color and of 9 females (7 of which were white, 2 brown with white spots) obtained in Utica, N. Y. The females of this strain are as good breeders as the average No. 8 or English, but do not grow up as fast as the latter.

Without tumors.	With tumors.
F_1 1 (1 III)	
F_2 11 (5 I 2 II 4 III)	3 (2 II 1 III)
F_3 19 (7 I 7 II 5 III)	16 (8 I 5 II 3 III)
F_4 10 (3 I 3 II 4 III)	7 (4 I 2 II 1 III)

Total:	
41 (15 I 14 II 12 III)	26 (12 I 9 II 5 III)
61% (36% I 35% II 29% III)	39% (46% I 35% II 19% III)
II + III 65% III 71%	II + III 35% III 29%

Disregarding mice that died during the first period of life without tumors, but including those acquiring tumors during this period, the figures are: without tumors 50% (54% II 46% III); with tumors 50% (46% I 35% II 19% III). The tumor rate in this strain is higher than that of No. 8 and 8½ and lower than that of English. The tumor age is slightly higher than in the case of the English and lower than in Nos. 8 and 8½. Wherever the figures are sufficiently large in the various generations, the results are similar.

6. Strain European.

Thirteen mice of various sizes were imported from Europe, Feb., 1910. They are on the whole of smaller size than the Granby mice. They and their offspring suffer greatly from mouse typhus. In Oct., 1912, their number has not yet increased. Two of the offspring of the imported stock have tumors. Only a relatively small number of this lot lived long enough to be included in the records.

Without tumors.	With tumors.
F_4 7 (5 I 2 II)	0
F_5 9 (5 I 4 II)	2 (2 I)
Total:	
16 (10 I 6 II)	3 (2 I 1 II)
84% (62% I 38% II)	16% (66% I 34% II)

Substrain European from Trio.

Two females and one male (all probably of one litter) were separated from the rest of the European mice; one of these two females had a tumor when not yet quite a year old (No. 228). The offspring of these mice were more healthy, but here also occasionally typhus appeared.

Without tumors.	With tumors.
F_2 5 (1 I 3 II 1 III)	1 (1 II)
F_3 18 (6 II 12 III)	2 (1 II 1 III)
F_4 49 (23 I 14 II 12 III)	11 (5 I 4 II 2 III)
F_5 7 (3 I 4 II)	1 (1 II)
Total:	
79 (27 I 27 II 25 III)	15 (5 I 7 II 3 III)
84% (34% I 34% II 32% III)	16% (33% I 47% II 20% III)
II + III 84%	II + III 16%

The same tumor rate occurs here as in the other European group, notwithstanding the much larger number of mice in this group. The figures in both substrains are therefore confirmatory of each other. The tumor rate is similar to that in No. 8½. The tumor age is somewhat lower than that of No. 8 and No. 8½. The results in the various generations agree fairly well with each other.

7. Strain German.

These mice were imported from Germany in May, 1910. They are somewhat smaller than the average Granby mice, but a little larger than the European. They are a very prolific strain, but again die early on account of disease. Three just weaned young females and one male were the only ones to thrive. All the mice recorded are the offspring of these four mice.

Without tumors.	With tumors.
F_1 0	3 (1 I 2 II or III)
F_2 0	2 (1 I 1 II or III)
F_3 6 (2 I 3 II 1 III)	3 (2 I 1 III)
F_5 0	2 (2 II)
F_6 4 (4 I)	
Total:	
10 (6 I 3 II 1 III)	10 (3 I 5 II 2 III)
50% (60% I 30% II 10% III)	50% (30% I 50% II 20% III)
II + III 36%	II + III 64%

The number of mice is not large in this strain, but the indications are that we have to deal with a strain rich in tumors, which, however, do not appear as early as is the case of the English. In this lot the mortality in early life was great. The tumor rate increases, therefore, if we exclude the animals dying in the first period of life.

8. Strain Heitler.

This strain is the offspring of mice imported more recently from Germany.

Without tumors.	With tumors.
F_1 13 (4 I 7 II 2 III)	3 (3 II)
81% (31% I 54% II 15% III)	19% (100% II)
F_2 39 (24 I 5 II 10 III)	9 (1 I 8 II)
81% (62% I 13% II 25% III)	19% (11% I 89% II)
F_3 13 (9 I 2 II 2 III)	11 (1 I 10 II)
54% (70% I 15% II 15% III)	46% (9% I 91% II)
F_4 9 (5 I 4 II)	5 (2 I 3 II)
64% (56% I 44% II)	36% (40% I 60% II)
Total:	
74 (42 I 18 II 14 III)	28 (4 I 24 II)
73% (56% I 24% II 20% III)	27% (14% I 86% II)
II + III 57%	II + III 43%

In this strain the tumor rate as a whole is similar to that of No. 8. The tumor age also is similar, inasmuch as the first period of life is poor in tumors, even more so than in Nos. 8 and 8½. It is an interesting fact that in this strain the large majority of tumors appear in the second period of life, while the third period is free from tumors. For this reason the tumor rate increases, if we omit from consideration the mice dying in the first period of life, just as we did in the case of No. 8. On the whole, the figures are within certain limits similar in the different generations.

9. Strain London.

This strain comprises about a dozen animals with various colors imported from London, England, late in the summer of 1911.

Of five imported females, four had tumors.

(a) Older records.

	Without tumors.	With tumors.
F_1	22 (7 I 5 II 10 III)	9 (4 I 2 II 3 III)
F_2	19 (8 I 7 II 4 III)	7 (1 I 3 II 3 III)
Total:		
	41 (15 I 12 II 14 III)	16 (5 I 5 II 6 III)
	72% (37% I 29% II 34% III)	28% (31% I 31% II 38% III)
	II + III 73% III 70%	II + III 27% III 30%

(b) Family of an imported London mouse with tumor (481).

F_1	4 (3 I 1 III)	0
F_2	1 (1 II)	0
F_3	1 (1 I)	1 (1 I)

(c) Later records (winter, 1914–15).

F_1	9 (1 I 3 II 5 III)	3 (3 III)
F_2	16 (7 I 4 II 5 III)	2 (1 II 1 III)
F_3	15 (9 I 5 II 1 III)	11 (3 I 8 II)
Total:		
	40 (17 I 12 II 11 III)	16 (3 I 9 II 4 III)
	54% (42% I 30% II 28% III)	26% (19% I 56% II 25% III)
	II + III 64% III 54%	II + III 36% III 26%

Thus the later records agree very well with the former one, as to the rate of tumors and tumor age. The irregularities found in the individual generations increase in inverse ratio to the number of the

animals observed; but on the whole the variations are not very great. The tumor rate and tumor age are similar to those of No. 8. Omitting animals in the first or in the first and second period of life does not greatly alter the result.

Total:	
87 (36 I 25 II 26 III)	33 (9 I 14 II 10 III)
73% (41% I 29% II 30% III)	27% (27% I 43% II 30% III)

II. Tumor Incidence and Tumor Age of Some Hybrids.

These and a number of other strains, not yet mentioned, were used for hybridization and have thus produced a considerable number of additional strains which we observed through successive generations. Inasmuch as we shall report on the crosses later in connection with another problem, we shall here cite only a few of them.

1. German + Carter.

(a) Early records.

Without tumors.	With tumors.
195 (56 I 57 II 82 III)	15 (2 I 3 II 10 III)
93% (29% I 29% II 42% III)	7% (13% I 20% II 63% III)

(b) Later records.

131 (23 I 31 II 77 III)	17 (2 I 8 II 7 III)
88% (17% I 24% II 59% III)	12% (12% I 47% II 41% III)

Total:	
326 (79 I 88 II 159 III)	32 (4 I 11 II 17 III)
91% (24% I 24% II 52% III)	9% (12% I 34% II 54% III)
II + III 90% III 89% I 95% I + II 92%	II + III 10% III 11% I 5% I + II 8%

Hence the earlier and later records are similar in regard to the tumor rate as well as to the tumor age. The tumors appear very late. In the successive generations some variations occur; they are, however, on the whole not very great; the deviation from the mean is greatest where the number of observed mice is smallest. The tumors appear in all generations at a late period of life.

F_1 11 (6 I 5 III)	3 (3 III)
78% (55% I 45% III)	22% (100% III)
F_2 71 (15 I 18 II 38 III)	3 (3 III)
96% (21% I 25% II 54% III)	4% (100% III)

F_3 205 (49 I 48 II 108 III)	24 (3 I 10 II 11 III)
90% (24% I 23% II 53% III)	10% (13% I 42% II 45% III)
F_4 39 (9 I 22 II 8 III)	2 (1 I 1 II)
95.1% (20% I 60% II 20% III)	4.9% (50% I 50% II)

2. Silver + 10.

Without tumors.	With tumors.
F_1 1 (1 III)	3 (1 II 2 III)
F_2 40 (19 I 15 II 6 III)	17 (3 I 7 II 7 III)
70% (48% I 37% II 15% III)	30% (18% I 41% II 41% III)
F_3 95 (31 I 34 II 30 III)	52 (20 I 17 II 15 III)
65% (33% I 35% II 32% III)	35% (38% I 33% II 29% III)
F_4 13 (4 I 1 II 8 III)	8 (3 I 4 II 1 III)
62% (31% I 7% II 62% III)	38% (38% I 49% II 13% III)
F_5 1 (1 III)	4 (4 III)
Total:	
150 (54 I 50 II 46 III)	84 (26 I 29 II 29 III)
64% (35 2/3% I 33 1/3% II 31% III)	36% (32% I 34% II 34% III)
II + III 62% III 61%	II + III 38% III 39%

The various generations behave in a similar manner as far as tumor rate and tumor age are concerned. The tumors appear at a medium age.

3. European + No. 10 (Nov. 3d Strain).

The tumors appear in the successive generations at a similar rate. Variations occur especially if the number of mice is relatively small in a certain generation. The tumors appear on the average at a medium age in the various generations.

Without tumors.	With tumors.
F_1 1 (1 III)	1 (1 III)
F_2 5 (3 I 2 II)	16 (6 I 10 II)
24% (60% I 40% II)	76% (37% I 63% II)
F_3 21 (9 I 6 II 6 III)	75 (16 I 36 II 25 III)
22% (43% I 28% II 29% III)	78% (21% I 48% II 31% III)
F_4 42 (30 I 12 II)	71 (33 I 30 II 8 III)
37% (71% I 29% II)	67% (46% I 43% II 11% III)
F_5 2 (1 I 1 II)	20 (8 I 5 II 7 III)
9% 50% I 50% II)	91% (40% I 25% I 35% III)
Total:	
71 (43 I 21 II 7 III)	183 (63 I 81 II 39 III)
28% (60% I 30% II 10% III)	72% (34% I 43% II 23% III)
II + III 19% III 15%	II + III 81% III 85%
I + II 31% I 41%	I + II 69% I 59%

The tumors appear not so early as in the case of the English. The tumor age is similar to No. 8.

III. Tumor Age.

In discussing the various strains of mice, we have already had occasion to mention the age at which the tumors appeared. We did not, however, express the frequency of tumors of a certain strain in relation to the number of mice alive at a certain period. This is done in the following pages. Here those strains which have a sufficient number of mice for the purpose of such a calculation are divided into three classes according to their age. We include here furthermore strains which were not mentioned previously. In each case we state how many mice were alive in each of the three periods and what percentage of the mice alive had tumors at that period of life. Thus we come to a more accurate determination of the frequency of tumors at a certain period of life and we can decide whether the various strains have the same tumor age and whether this tumor age is correlated with other factors. The figures which we thus obtain are only an approximation to the real conditions; they are not absolutely correct, because in computing the figures we ignored the fact that during the various periods mice died at different times, while we assumed for the purpose of obtaining a relatively small number of comparable figures in each strain, that they lived to the end of that period of life. However, we believe that this inaccuracy does not interfere seriously with a representation of the actual facts.

In defining the tumor age of the various strains we had further to consider the fact that the figures obtained stating the per cent of tumor mice living at a certain period depended also on the absolute frequency of tumors of a certain strain and not only on the distribution of tumors according to the age of the animals. In order to eliminate the former factor it was necessary to determine in all strains the relation between the percentage of tumor mice in each of the three periods of life.

On the following pages we state in each strain the number of tumor mice expressed in per cent of living mice in each period.

We then state the frequency of tumors in the strain as a whole, and then follows the relation between tumor rate in the first and second plus the third periods, and the relation between tumor rate in the first and the second periods, between tumor rate in the second and third, and lastly between the tumor rates in the first plus the second and third periods of life. Thus we obtain an insight into the relative frequency of tumors in the three different periods of life.

European + No. 10 (Nov. 3d strain).

I	Period	254	mice	63	tumors	= 25%
II	"	148	"	120	"	= 81%
III	"	46	"	39	"	= 88%

72% I : II+III = 1 : 7 I : II = 1 : 3
II : III = 1 : 1 I+II : III = 1.2 : 1

English A.

I	Period	175	mice	52	tumors	= 29%
II	"	80	"	51	"	= 64%
III	"	16	"	8	"	= 50%

63% I : II+III = 1 : 4 I : II = 1 : 2.2
II : III = 1.3 : 1 I+II : III = 1.9 : 1

English Sable.

I	Period	252	mice	115	tumors	= 46%
II	"	88	"	61	"	= 69%
III	"	21	"	13	"	= 62%

70% I : II+III = 1 : 2.8 I : II = 1 : 1.5
II : III = 1 : 1 I+II : III = 1.9 : 1

English except Silver. Total:

I	Period	537	mice	219	tumors	= 41%
II	"	203	"	146	"	= 72%
III	"	38	"	22	"	= 58%

68% I : II+III = 1 : 3.2 I : II = 1 : 1.7
II : III = 1.3 : 1 I+II : III = 2 : 1

English Silver.

I	Period	117	mice	8	tumors	= 6.1%
II	"	69	"	4	"	= 5.1%
III	"	16	"	0	"	= 0 %

7% I : II+III = 1.2 : 1 I : II = 1.2 : 1

No. 8.

I	Period	268	mice	24	tumors	= 8%
II	"	150	"	57	"	= 38%
III	"	73	"	27	"	= 37%

30% I : II+III = 1 : 9.3 I : II = 1 : 4.7
II : III = 1 : 1 I+II : III = 1.2 : 1

No. 8½.

I	Period	158	mice	6	tumors	= 4%
II	"	109	"	17	"	= 15%
III	"	40	"	4	"	= 10%

17% I : II+III = 1 : 6.2 I : II = 1 : 3.7
II : III = 1.5 : 1 I+II : III = 1.9 : 1

Carter.

I	Period	67	mice	12	tumors	= 17 %
II	"	40	"	9	"	= 22.5%
III	"	17	"	5	"	= 28 %

39% I : II+III = 1 : 3 I : II = 1 : 1.3
II : III = 1 : 1.2 I+II : III = 1.4 : 1

German.

I Period	20 mice	3 tumors	= 15%	50%	I : II+III = 1 : 7.4	I : II = 1 : 3
II "	11 "	5 "	= 45%		II : III = 1 : 1.5	I+II : III = 1 : 1.1
III "	3 "	2 "	= 66%			

European.

I Period	94 mice	5 tumors	= 5%	16%	I : II+III = 1 : 4.2	I : II = 1 : 2.2
II "	62 "	7 "	= 11%		II : III = 1.1 : 1	I+II : III = 1.6 : 1
III "	28 "	3 "	= 10%			

Heitler.

I Period	102 mice	4 tumors	= 4%	27%	I : II+III = 1 : 10.7	I : II = 1 : 10.7
II "	56 "	24 "	= 43%		II : III ∞ I+II : III ∞	
III "	14 "	0 "	= 0%			

London.

I Period	120 mice	9 tumors	= 7%	27%	I : II+III = 1 : 6.6	I : II = 1 : 2.7
II "	75 "	14 "	= 19%		II : III = 1 : 1.4	I+II : III = 1 : 1
III "	36 "	10 "	= 27%			

European + No. 10 (Nov. 8 strain).

I Period	96 mice	20 tumors	= 21%	65%	I : II+III = 1 : 6.1	I : II = 1 : 2.6
II "	61 "	33 "	= 54%		II : III = 1 : 1.4	I+II : III = 1 : 1
III "	12 "	9 "	= 75%			

Cream + 10.

I Period	174 mice	4 tumors	= 3%	36%	I : II+III = 1 : 17	I : II = 1 : 5
II "	152 "	23 "	= 15%		II : III = 1 : 2.4	I+II : III = 1 : 2
III "	96 "	35 "	= 36%			

No. 8 + German.

I Period	244 mice	21 tumors	= 9%	41%	I : II+III = 1 : 6.8	I : II = 1 : 3.1
II "	183 "	51 "	= 28%		II : III = 1 : 1.2	I+II : III = 1 : 1.1
III "	86 "	28 "	= 33%			

101 + 103.

I Period	152 mice	6 tumors	= 4%	34%	I : II+III = 1 : 17	I : II = 1 : 7.2
II "	97 "	27 "	= 29%		II : III = 1 : 1.4	I+II : III = 1 : 1.2
III "	45 "	18 "	= 40%			

German + Carter.

I Period	358 mice	4 tumors	= 1%	9%	I : II+III = 1 : 14	I : II = 1 : 4
II "	275 "	11 "	= 4%		II : III = 1 : 2.5	I+II : III = 1 : 2
III "	176 "	17 "	= 10%			

European Hybrid + 8½ F_4.

I Period	473 mice	8 tumors	= 2%	16%	I : II+III = 1 : 13	I : II = 1 : 2.5
II "	364 "	19 "	= 5%		II : III = 1 : 4.2	I+II : III = 1 : 3
III "	233 "	50 "	= 21%			

Silver + 10.

I Period	234 mice	26 tumors	= 11%	36%	I : II+III = 1 : 5.2	I : II = 1 : 1.6
II "	154 "	29 "	= 18%		II : III = 1 : 2.2	I+II : III = 1 : 1.4
III "	75 "	29 "	= 39%			

(103 + European) F_1 + III. Daughter of No. 10.

I	Period	168	mice	3	tumors =	2%
II	"	115	"	11	"	= 9%
III	"	67	"	14	"	= 21%

17% I : II+III = 1 : 15 I : II = 1 : 4.5 II : III = 1 : 2.2 I+II : III = 1 : 3

European 151 + 8 F_5

I	Period	125	mice	8	tumors =	6%
II	"	86	"	20	"	= 23%
III	"	47	"	9	"	= 19%

30% I : II+III = 1 : 7 I : II = 1 : 3.8 II : III = 1.2 : 1 I+II : III = 1.5 : 1

European + 102. 103.

I	Period	146	mice	2	tumors =	1.5%
II	"	109	"	9	"	= 8 %
III	"	76	"	19	"	= 25 %

21% I : II+III = 1 : 22 I : II = 1 : 5.3 II : III = 1 : 3.1 I+II : III = 1 : 2.6

London + (European + 103) F_3.

I	Period	78	mice	0	tumors =	0%
II	"	66	"	2	"	= 3%
III	"	33	"	2	"	= 6%

5% II : III = 1 : 2

Cream.

I	Period	177	mice	0	tumors =	0%
II	"	120	"	2	"	= 2%
III	"	53	"	2	"	= 4%

2.3% II : III = 1 : 2

8½ + English Sable.

I	Period	48	mice	20	tumors =	41%
II	"	15	"	9	"	= 60%
III	"	1	"	0	"	= 0%

61% I : II+III = 1 : 1.5 I : II = 1 : 1.5

8½ + No. 10 F_1 (Nov. 8 strain).

I	Period	82	mice	6	tumors =	7%
II	"	68	"	19	"	= 27%
III	"	35	"	15	"	= 43%

49% I : II+III = 1 : 10 I : II = 1 : 4 II : III = 1 : 1.6 I+II : III = 1 : 1.3

(8½ + No. 10) + No. 10 (Nov. 8).

I	Period	72	mice	5	tumors =	7%
II	"	48	"	9	"	= 19%
III	"	20	"	12	"	= 60%

36% I : II+III = 1 : 11 I : II = 1 : 2.7 II : III = 1 : 3.2 I+II : III = 1 : 2.3

Michigan Wild + 101 F_2.

I	Period	50	mice	13	tumors =	26%
II	"	23	"	9	"	= 39%
III	"	10	"	7	"	= 70%

58% I : II+III = 1 : 4 I : II = 1 : 1.5 II : III = 1 : 1.8 I+II : III = 1 : 1.1

121 (English Tan) + Cream.

I	Period	92	mice	16	tumors =	7%
II	"	56	"	18	"	= 31%
III	"	16	"	5	"	= 31%

42% I : II+III = 1 : 8.8 I : II = 1 : 4.4 II : III = 1 : 1 I+II : III = 1.2 : 1

European + 146 (English Tan).

I	Period	74	mice	3	tumors =	4%
II	"	58	"	13	"	= 22%
III	"	22	"	5	"	= 23%

28% I : II+III = 1 : 11 I : II = 1 : 5.5 II : III = 1 : 1 I+II : III = 1.1 : 1

English 121 + German.

I	Period	39	mice	10	tumors	= 25%	49% I : II+III=1 : 3.6 I : II=1 : 1.6 II : III=1 : 1.3 I+II : III=1.3 : 1
II	"	18	"	7	"	= 39%	
III	"	4	"	2	"	= 50%	

White English + (8 + German) F_4.

I	Period	82	mice	27	tumors	= 33%	63% I : II+III=1 : 2.2
II	"	33	"	25	"	= 73%	
III	"	1	"	0	"	= 0%	

We may arrange the various strains into four classes according to the relative frequency of tumors in the various periods of life. While there are some slight variations within each class, on the whole the various strains in each class behave in a similar manner.

Class I. Early Tumors.

I : II + III = 1 : 2.8 I : II = 1 : 1.6 II : III = 1 : 1.2 I + II : III = 1.5 : 1

English 68%.
English Silver 7%.
8½ + English Sable 61%.
English (121) + German 49%.
White English + (8 + German) F_4 63%.
Michigan Wild + 101 F_2 (in Period III, more tumors) 58%.
Carter 39%.
European 16%.

Average tumor rate 45%.

Class II. Tumors of Medium Age.

I : II + III = 1 : 7.4 I : II = 1 : 3.5 II : III = 1 : 1.2 I + II : III = 1.2 : 1.

Nov. 3 strain 72%.
Nov. 8 strain 65%.
No. 8 30%.
No. 8½ 17%.
Heitler (in II period very high tumor rate) 27%.
London 27%.
European + 146 (English Tan), (not quite as good, in Period I, less tumors) 28%.
No. 8 + German 41%.
Silver + 10 (less tumors in Period III, more in II, nearer Class I) 36%.
European Hybrids 151 + 8 F_5 (Period I low, Period II high, Period III lower, somewhat better than Class II) 30%.
121 + Cream 42%.
German 50%.

Average tumor rate 38%.

Class III. Great Frequency of Tumors in Period III.

I : II + III = 1 : 10.5 I : II = 1 : 3.3 II : III = 1 : 2.4 I + II : III = 1 : 1.8.

8½ + No. 10 F_1 (Nov. 8 strain) 49%.
(8½ + No. 10) + No. 10 (Nov. 8 strain) (in Period III many tumors) 36%.
Average tumor rate 42½%.

Class IV. Late Tumors.

I : II + III = 1 : 16.3 I : II = 1 : 4.7 II : III = 1 : 2.6 I + II : III = 1 : 2.3.

Cream + 10 36%.
Cream 2.3%.
101 + 103 = [101 + (103 + European)] 34%.
German + Carter 9%.
European Hybrid + 8½ F_4 = [(European + 102) + 8½ F_4] 16%.
Hybrids (103 + European) F_1 + III, daughter of No. 10 17%.
European + 102, 103 21%.
London + (European + 103) F_3 5%.
Average tumor rate 17.5%.

In Class I, in which the tumors appear relatively early, we find in the combined second and third periods of life 2.8 times as many tumors as in the first period; in the second period of life 1.6 times as many tumors as in the first period; in the third period 1.2 times as many tumors as in the second period; and in the combined first and second periods 1.5 times as many tumors as in the third period. While, therefore, in this class the tumors are more frequent in the second period than in the first, and in the third than in the second, the differences in the percentages are not very marked. The tumors appear here relatively early, the percentage of tumors in the first period of life being considerable. We include in this group eight strains, counting the various English substrains as one strain, with the exception of the Silver. We added to the name of each strain the number giving the frequency of tumor mice in per cent of the total number of mice, irrespective of the age at which they appear. If we designate as a high tumor rate 40 to 100 per cent, as a medium tumor rate 20 to 40 per cent, and as a low tumor rate 0 to 20 per cent, we find that five of the eight strains have a high tumor rate, one a medium tumor rate (with a figure near the lower border of the high tumor rate), and two a low tumor rate. Of these two, one

strain (Silver) is related to the other English strain, which has a high tumor rate. We see therefore that the tumor age can vary independently of the tumor frequency. English Silver has like the other English strain an early tumor age, but in contradistinction to the other English mice a very low tumor rate. The average tumor rate in Class I is 45 per cent. It would be higher if we had counted the various English strains separately.

In Class II the tumors in the second period are 3.5 times more frequent than in the first period; the tumors in the second period are therefore here relatively (compared to the frequency in the first period) considerably more frequent than in the first class. In the third period the tumors are 1.2 times more frequent than in the second period; this is the same proportion between the frequency in the third and second periods as in the first class; but absolutely the tumors are here more frequent in the third period; 11 strains belong to this class; 4 of these have a high, 6 a medium, and 1 a low tumor rate. The average tumor rate is 38 per cent.

Class III consists of only two strains, which as far as the relation between the number of tumors in the first and second period of life is concerned, stand between the first and second class, but are nearer the second class, in which, however, the relative frequency of tumors in the third period is greater than in the second class. The average tumor rate is here 42.5 per cent.

Class IV contains the late tumors. The relative preponderance of the frequency of tumors in the second over that in the first period is here greater than in the first, second, or third class, and again the preponderance of the third period over the second period is greater than in the former classes. Eight strains are included in this class. None of these show a high tumor rate, 2 show a medium, and 6 a low tumor rate. The average tumor rate in this class is 17.5 per cent.

We may draw from this analysis the following conclusions:

1. There exists a certain relationship between tumor frequency and tumor age. On the whole, the more frequent the tumors, the earlier they appear in the various strains. It might be conceivable that the frequency of the tumors was independent of the tumor age;

that in strains in which the tumor frequency is greater, the tumors appear in the same percentage in the various periods of life, but this is evidently not the case. In strains in which the tumor frequency is greater, they appear on the whole also at an earlier period of life.

2. This parallelism between tumor frequency and tumor age is, however, not complete. The tumor age seems to be as characteristic for a strain as the tumor rate. In strains with a similar rate of frequency, the tumor age may be different. This difference is probably not accidental, because (1) if we have substrains related to each other, the tumor age is usually similar in all of them, and (2) the tumor age of the constituent strains seems to influence the tumor age of the crosses. How far this latter relation holds good, we shall discuss in another communication. On the other hand, we found in the case of the substrain Silver that it had a similar tumor age to the English strain, although the tumor rate of the Silver is considerably lower than that of the other English strains.

3. The tumor age is transmitted from generation to generation in a similar manner to the tumor rate. We may therefore conclude that in all probability tumor rate and tumor age represent distinct unit factors which frequently, but not in all cases, are in some way linked to each other.

4. We may furthermore conclude that the age where the maximum of tumors occurs varies in different strains. While in some it appears in the second period of life, in others it is in the third period. Here again the maximum is on the whole reached at an earlier period of life in strains with a high tumor rate. But here also peculiarities exist in different strains.

SUMMARY.

1. It is possible to split a strain of mice into certain substrains in which the tumor incidence is in some way linked to the color of the mice. Thus we could split off from the English strain, which as a whole and in various substrains with mixed colors (English A and Sable) has a high tumor rate, substrains with light tan color and pink eyes (101 and Tan) which have a high tumor rate like the large majority of the English mice, and two other apparently

recessive strains breeding true, which have a very low tumor incidence (Silver and Silver Fawn). Therefore, certain combinations of factors which determine certain colors of mice determine at the same time the tumor incidence of these strains or substrains. In the majority of cases isolated families bred through several generations separately from the majority of the other substrains give approximately the same tumor rates as the others; in some cases, however, it may perhaps be possible to separate from the main strain a family with a different rate.

2. The tumor incidence and the tumor age found in the earlier periods of our work are approximately the same as in the more recent period. On the whole, the results obtained in successive generations of the same strain also agree well with each other; the results are fairly constant; the deviations which occur are in most cases due to the small number of animals observed in the certain generations. Discarding all the mice dying in the first or in the first and second periods of life usually does not alter essentially the tumor ratio of a certain strain.

3. A certain relationship exists between tumor frequency and tumor age. On the whole, the more frequent the tumors, the earlier they appear in the various strains. This parallelism between tumor frequency and tumor age is, however, not complete. The tumor age seems to be as characteristic for a certain strain as the tumor rate. Certain substrains which differ in tumor frequency may show approximately the same tumor age. Strains with similar tumor frequency may show a different tumor age. We may therefore conclude that in all probability tumor rate and tumor age represent distinct unit factors, which are frequently, but not in all cases, linked in some manner to each other.

4. The age at which the maximum of tumors appears varies in different strains. The maximum may fall into the second or third period of life. On the whole, the maximum is reached at an earlier period of life in those strains which have a high tumor rate. But here also peculiarities exist in different strains.